SPIROCHAETAL JAUNDICE.

By

George Buchanan, M.B., Ch.B., D.P.H..

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CONTENTS.

I. INTRODUCTION AND HISTORICAL SURVEY.

Various forms of infective jaundice have long been recognised, but the cause in certain of them still remains obscure. One of these, described by Weil in 1886, was subsequently known by his name, but when the causal agent,(spirochaeta or leptospira icterohaemorrhagiae), was discovered the name "Spirochaetosis Icterohaemorrhagica" was substituted, and this led to the reporting of similar cases in many countries. It was not, however, until 1923 that the occurrence of the disease in Scotland was suspected - a suspicion which was confirmed in the following year.

During the year 1923 there occurred among miners working in certain coal-mines in East Lothian, an outbreak of jaundice of unknown origin. From the clinical features spirochaetal jaundice was suspected. Several of the affected miners were admitted to the Royal Infirmary, Edinburgh, and the present writer was given the opportunity of investigating the eighth suspected case received into hospital towards the end of/

of November 1923. The patient was under the care of Professor G. Lovell Gulland who kindly granted every facility for the investigation of the condition, which was subsequently diagnosed as spirochaetosis icterohaemorrhagica. This was the first recorded instance of the disease in Scotland, and owing to the interest and industrial importance of the discovery an investigation into the aetiology, symptomatology, and pathology of the condition was undertaken. The results of this investigation form the subject matter of this thesis.

The patient referred to, a young miner, was admitted into hospital on the 5th day of illness when jaundice was evident. Specimens of blood and urine were examined for spirochaetes, and according to the reports issued the results were negative. At a later date a further specimen of urine was examined by the writer, but again no definite spirochaetes were recognised. On enquiry it was found that the urine had stood overnight; it was therefore thought advisable not only to obtain a fresh specimen but also to see the patient, and to ascertain particulars of the illness personally. It so happened that this sample/

sample was collected on the 30th day of illness; it was immediately centrifuged, wet preparations were examined by dark-ground illumination, and a guinea pig received an intraperitoneal inoculation of 2cc. of urine with deposit. By the dark-ground microscope a few forms suggestive of spirochaetes were observed; stained films however showed forms definitely recognisable as spirochaetes. The guinea pig injected with the urine died 14 days after inoculation; it showed marked jaundice, subcutaneous and internal haemorrhages, and an organism corresponding morphologically to leptospira icterohaemorrhagiae was demonstrated in the tissues, (vide Section III, d., p. 66). Cultivation of the spirochaete from these tissues by the method described in Section III, 4., p. 76 was successful. In this manner the cause of the outbreak of jaundice amongst miners in East Lothian was proved to be of spirochaetal origin. This interesting result directed attention first to the question of a local source of infection, and as rats had proved to be a source of infection in other countries, arrangements were made to obtain a few from the coal-mines in which cases of jaundice had occurred. Three rats were received at this period; in two of these leptospirae were found in the kidney, and inoculation/

inoculation of an emulsion of this organ into guinea pigs produced the typical manifestations of the disease. The fact that rats in Scotland harboured the infective agent was thus established for the first time. Following on these initial findings, the investigation was extended to a study of the incidence and circumstances of the disease as it occurs in Scotland generally.

Historical.

The condition known as jaundice has been described since ancient times, and attention has been drawn by Cockayne to its mention in "De internis affectionibus", often ascribed to Hippocrates. The epidemic form of jaundice appears to have been first recorded by Cleghorn, who reported its prevalence in Minorca in 1745. In subsequent years records indicate that many epidemics, characterized by jaundice, occurred amongst the civilian populations in Europe, and amongst troops whenever they were concentrated. The disease is referred to by Blumer as having occurred in America during the war of 1812, and reports exist of repeated outbreaks in the United States from 1857 onwards. Many French writers have described epidemics of jaundice, amongst whom may be mentioned Ozanam (1846-9), Monneret/

Monneret (1859), Laveran (1865), Larrey (1880), Lancereaux (1882), Landouzy (1883), and Mathieu (1886). Various names were applied by the different authors such as "Ictere grave essential" by Lancereaux, "Fievre bilieuse" by Landouzy, while Mathieu named it "Ictere febrile a rechutes", on account of the tendency to relapses in his cases. In Germany epidemic jaundice has been referred to as "Biliöse Typhoid", "Icterus Typhosus" and "Typhus biliosus nostras". The outbreaks in Europe are stated to have occurred most frequently amongst young adults, particularly soldiers, butchers, and sewermen. As the aetiological factor, however, in these epidemics remained obscure, and the reports by the various authors vary, diverse opinions have now been expressed regarding the aetiological nature of the various epidemics, and it is thought probable that two distinct types, or distinct groups of disease have been confused, i.e. (1) epidemic jaundice and (2) catarrhal jaundice.

As certain of the accounts indicate a close relationship to the type of jaundice considered in this thesis, reference to these may appropriately be made. Carville (1859) described an epidemic of jaundice in the garrison at Gaillon, in which, out of 47 persons affected, 11 died. In all the cases jaundice and albuminuria were associated, and haemorrhages were common/

common; epistaxis occurred in 15, purpura in 3, and one had haematemesis. The incubation period is stated to have been six days. An outbreak of a similar nature was recorded by Worms (1865), at St Cloud. In 1880 Larrey described an epidemic of jaundice which occurred during the siege of Cairo, and noted particularly the association of haemorrhages with the disease. Referrence is made by Dawson, Hume and Bedson, to cases of jaundice reported in 1886 by Mathieu, who considered that the fever, general symptoms, enlargement of the spleen, and albuminuria justified the name of infectious jaundice, the designation of catarrhal jaundice being inadequate. Some time later in 1886 Weil published an account of four cases of infectious jaundice in two of which febrile relapses were described. Subsequently the disease presenting a symptom-complex of fever, jaundice, enlargement of liver and spleen, the occurrence of haemorrhages and occasionally febrile relapses, has frequently been referred to as Weil's disease. Although the cause in no instance was proved, attention was drawn by several writers to probable sources of infection, which are noteworthy in the light of recent work on the ætiology of the disease. Worms attributed the source of infection in the St Cloud epidemic in 1865 to the drinking water obtained from a polluted reservoir: the incidence of the disease abated when the water from this source ceased/

ceased to be used. Uhlenhuth and Zuelzer refer to observations made by an army medical officer in Germany on epidemics of jaundice amongst troops in 1873 and 1874. It was noted that the most numerous cases of jaundice occurred amongst those troops which enjoyed bathing facilities, and that other corps, denied this privilege, remained free from the disease. The bathing was performed in the Elbe below the point of discharge of the town effluents into the river from open drains. The discharged substances polluted the beach and caused a black slime to form which was evident at low water. The origin of the disease was attributed to the circumstance, that in bathing and diving the slime would be disturbed and the specific organism would probably be swallowed by the bathers. In 1876 the bathing station was changed to a part of the river above the entrance of the town effluent and a remarkable decrease in the number of cases resulted. It was also observed that of the troops which bathed in another arm of the Elbe, none contracted the disease. Similar observations were made on different occasions by later German writers. Amongst other epidemics of jaundice attributed to sewage is that described by Valassapoulo in 1908 in Alexandria. Those affected lived in most insanitary surroundings close to where sewage was discharged by open drains into the sea.

In Nauplia epidemics of jaundice are stated to have ceased since the drainage was improved. Mention may here be made of a report on a severe case of "ictere infectieux primitif", so named by Widal and Abrami (1908), because the clinical and pathological features described, are exactly similar to those of spirochaetal jaundice. In none of these epidemics or sporadic cases of infective jaundice, however, was the actual cause disclosed, which still leaves the question of the spirochaetal origin of these earlier outbreaks a matter of controversy. Within comparatively recent years, however, the cause of one form of infectious jaundice has been definitely proved.

A disease considered similar to Weil's disease in Europe has been prevalent in Japan for many years. In November 1914, Inada and Ido discovered a spirochaete in the liver of a guinea pig inoculated with blood from a patient suffering from the Japanese form of Weil's disease. After further work they proved the specificity of the new spirochaete, for which they proposed the name "Spirochaeta Icterohaemorrhagiae". The work of the Japanese authors will be more fully discussed in Section III., 1., but reference may here be made to the title of "Spirochaetosis Icterohaemorrhagica", which they introduced as a substitute for the/

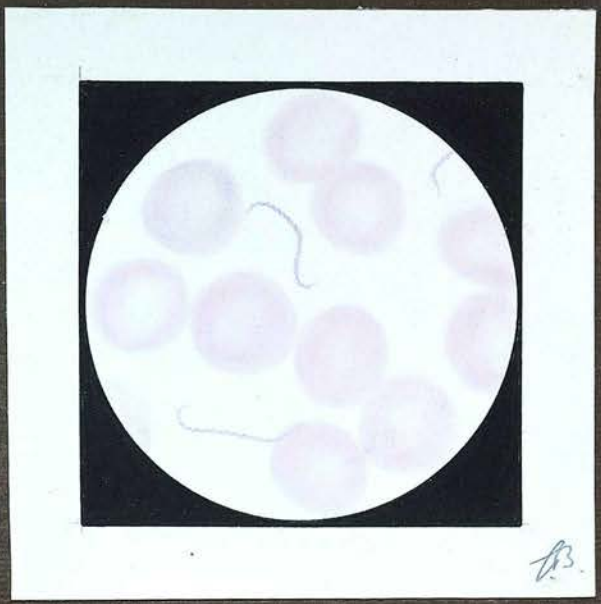


Fig. 1. Spirochaeta or leptospira icterohaemorrhagiae in blood of infected guinea pig. Giemsa's stain.
To show the distinctive morphology of the organism viz. the presence of very fine spirals and incurved ends. Noguchi (1917) considered it to be strikingly different from any other spir-
-ochaete hitherto described, and created a new genus, Leptospira, the type species of which is represented by this organism.
The views of other writers on this point of nomenclature are discussed in a later section (p.73).

the various names applied to this disease. In 1915 Uhlenhuth and Fromme, as well as Hübener and Reiter, announced that the Weil's disease which occurred amongst German soldiers during the Great War, could be communicated to guinea pigs by inoculation of the patients' blood. In a later publication (1916) Hübener and Reiter noted the finding of a spirochaete in the organs of the inoculated animals, and named it "Spirochaeta Nodosa" without reference to the earlier Japanese work. Subsequent to the publication in 1916 of an English version of the work of the Japanese authors, Inada, Ido, Hoki, Kaneko and Ito, the disease became more widely recognised, particularly amongst the troops engaged on the Western front in 1916. Stokes and Ryle announced the existence of the disease amongst British soldiers in Belgium. The diagnosis was confirmed by transmitting the disease to guinea pigs by inoculation of the patient's blood and by demonstrating in the animal tissues a spirochaete now considered identical to spirochaeta icterohaemorrhagiae. Amongst the French soldiers on the Western front the prevalence of the disease was similarly confirmed by Costa and Troisier, Garnier and Reilly, Renaux, Merklin and Licust, Ameuille, Salmon and /

and Neveu, and several other observers. The spirochaete isolated from the French cases was also considered to be very similar to the Japanese type, with the exception of that found in a certain outbreak in the Maritime Hospital at L'Orient. The existence of the disease on the Italian front was reported by Monti, and later by Sisto, in 1917. At Salonica a form of jaundice occurred amongst the Canadian soldiers which was suspected, though not proved, to be of spirochaetal origin.

Since the end of the War in 1918, epidemics and sporadic cases of spirochaetal jaundice have been reported by writers in many countries. Reiter (1919), and Uhlenhuth and Zuelzer (1922) published accounts of outbreaks in Germany. In 1922 the disease in sporadic form was described by Schürer in Germany, Manson-Bahr in England, Guiteras in Havana, and Grapiolo in the Argentine. In 1923 Passey and Ryle gave notes on a probable case in Surrey, and Lepidus and Flaun first recorded the disease in Sweden. In 1924 Carbo-Neboa described a case in Ecuador, and outbreaks of spirochaetal jaundice were reported in Sumatra by Baermann, in Scotland by Gulland and Buchanan, and in Northern France/

France by Railliet and the French public health authorities. In 1925 Lyon and Buchanan described another small outbreak in Scotland, and epidemics amongst school children have also been recorded by Körner in Germany, and Hindle and Brown in England.

II. CLINICAL INVESTIGATION.

This enquiry was begun in December 1923 and since that time the writer has investigated 51 cases of suspected spirochaetal jaundice in Scotland. Previous to this date in 1923 the infection was considered to be the cause of illness in nine miners, solely on account of the clinical symptoms. Spirochaetal jaundice was excluded in 29 out of the 51 cases examined, on both clinical grounds and negative laboratory results. The disease was diagnosed in the remaining 22 mainly on the manifestation of certain clinical features along with the presence of spirochaetes in the urine, and confirmed in some instances by transmitting the disease to animals inoculated with the urine. Attempts to establish a diagnosis by means of the examination and the animal inoculations of suspected blood was not successful in any case. The reason for this failure is attributed to the fact that the disease in most of the/

the patients was not suspected or reported until the infective period of the blood had passed. This mode of diagnosis is more fully discussed in Section V,1. (p.124)

1. Clinical History.

Although advantage has also been taken of opportunities to study the clinical course of the disease in many of the patients, it is not intended to discuss this aspect at length; but a brief description of a case representative of the milder form in Scotland may not be inappropriate.

> The patient, a miner aged 18, while walking home from his work felt chilled, and had headache and abdominal pain. On the same day nausea developed and he vomited a small quantity of brownish fluid - suggestive of a haematemesis. Marked prostration developed rapidly, with general muscular pains and continuation of the headache. Epistaxis occurred and diarrhoea was succeeded by constipation/

constipation. Jaundice, stated to have been noticeable on the fourth day, was well marked on his admission to hospital on the sixth day of illness. The conjunctivae were injected, the lips dry, and the tongue was furred and brown. He was weak, listless and disinclined for food. Temperature 101°F., pulse 84, respirations 32. The heart and lungs were normal. The notable features of the physical examination were the tenderness over the liver region and the muscles generally; the liver was palpable and later reached to 1½ in., below the costal margin. The spleen was not enlarged. The urine contained bile and a small quantity of albumin; red corpuscles, and a few granular and epithelial casts were present microscopically. A blood count showed no diminution of red cells, the white cell count was 10,800 per cm.m. and the haemoglobin 70 per cent. The faeces gave a definite blood reaction and bile was present. The temperature fell by lysis to 99°. - 98° in twelve days, and the jaundice gradually declined from about the twelth day, but did not entirely clear up until the beginning of the fifth week. Spirochaetes were/

were found in the urine during the fourth week of illness.

A rise of temperature of very remittent type occurred at the end of the third week and lasted eight days - 103°F was recorded on one evening. There was no exacerbation of symptoms during this febrile period, after which the patient made a good recovery.

This case shows a fairly typical history of the disease in its milder form, the noteworthy features being, sudden onset with headache, body pains, gastro-intestinal symptoms and high fever, the occurrence of slight epistaxis, the appearance of jaundice about the fifth day, the fall of temperature at the end of eleven or twelve days, and the finding of the specific spirochaete in the urine.

These features, characteristic of spirochaetal jaundice as it occurs in Scotland, agree in the main with accounts of the disease in other countries. The only minor variation, compared with the cases in Belgium described by Dawson, Hume, and Bedson, and also Ryle, consisted in the occurrence of haemorrhagic herpes/

Fig. 2

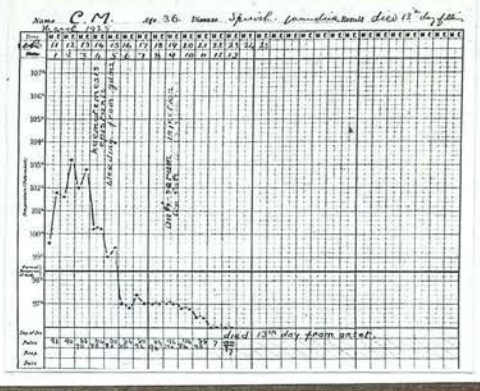
SPIROCHAETAL JAUNDICE. Severe Type.

Case C.M. age 36, aluminium worker. Works situated near a rat infested refuse dump where C.M. worked. Rats found on examination to be infected with leptospirae.

March 11th Complained of aches and pains all over body.
" 12th. Vomited several times and felt very exhausted. Slight jaundice noticed 13th.
" 14th Haematemesis, epitaxis, and bleeding from gums: jaundice more pronounced.
" 16th Intense jaundice over face and body, conjunctivae markedly injected. Tongue dry, brown, and furred. Petechial haemorrhages numerous on body and extremeties, particularly shoulders, abdomen and round ankles. Haemorrhages distinctly raised, some ¼in. in diameter. Liver enlarged, 1in. below costal margin Spleen not palpable. Muscles tender, especially calves of legs.

Blood Count: R.B.C. 4,800,000. W.B.C. 12,400. Polymorph-leucocytosis.

March 18th Herpes on lips and left upper eyelid becoming haemorrhagic.
" 20th Incontinence faeces and urine, drowsy, soft pulse.
" 22nd Very little urine passed.
" 23rd Slight bleeding from gums, difficulty in swallowing, pulse imperceptible, twitching of face and jerking of head, suppression of urine. Died 8·40 p.m..
Anti-serum was administered on first seeing the patient on the 9th day of illness, but this was too late to be effective.



herpes labialis in only 5% of the patients in Scotland, while these authors described 40 - 43% of their cases with perpes labialis invariably haemorrhagic. The occurrence of relapses or small pyrexial recrudescences, as in the case cited, was noted in 10% compared with 18% of Ryle's cases. According to Stokes, Ryle and Tytler, the disease may occur without icterus. In such cases Costa and Troisier state that it may present itself in the form of a simple or relapsing meningitis, and inoculation of the cerebro-spinal fluid into guinea pigs may reproduce the disease.

2. Clinical Pathology.

In addition to blood examination for spirochaetes and inoculation of guinea pigs with suspected blood, methods which will be considered later, blood counts were done in some of the cases. In the severe forms there was slight anaemia with reduced haemoglobin percentage, 70 - 90 per cent, and a variable leucocytosis up to 20,000 cm.m.. The differential count showed an increase only in the polymorpho-nuclear leucocytes up to about 84 per cent. The urine was examined for spirochaetes but it may be mentioned here that a diagnosis based on urinary findings alone presented difficulties owing to factors considered in Section V. (p.126).

The following table sets forth certain features associated with the twenty two cases investigated.

TABLE I.

Showing the incidence of two important clinical manifestations, the results of urinary examination and animal inoculations with urine, and the severity of the disease in each case.

Case	Jaundice	Haemorrhages	Spirochaetes found in urine	Result of Animal Experiments	Severity.
1. T.W.	+	+	+	+	Mild
2. A.McN.	+	−	+	−	Slight
3. T.B.	+	+	−	−	Slight
4. R.C.	+	−	+	−	Slight
5. A.D.	+	−	−	−	Slight
6. B.W.	+	+	+	L	Fatal
7. R.E.	+	+	+	−	(Mod.- (Severe
8. D.B.	+	−	+	−	Mild
9. N.B.	+	+	+	L	Mild
10. M^{t}B.	+	+	+	−	V. Severe
11. M.B.	+	−	+	+	Mild
12. J.K.	+	−	+	−	Mild
13. M.Br.	+	+	+	−	Mild
14. G.B.	+	+	+	L	Slight
15. R.B.	+	+	+	L	(Mod. - (Severe
16. A.C.	+	+	+	L	Fatal
17. J.L.	+	−	−	L	Mild
18. J.M.	+	+	−	L	Fatal
19. M.Mc.	+	−	+	L	Mild
20. A.McD.	+	−	+	−	Slight
21. C.M.	+	+	+	−	Fatal
22. J.R.	+	+	no specimen obtained	−	Fatal

L = lung haemorrhages present in guinea pigs inoculated, but no jaundice obvious and no spirochaetes found (vide p. 129).

In the nine suspected cases of the disease which occurred amongst miners previous to this investigation, there is a record of jaundice in all, the occurrence of haemorrhages in four, and death in three.

Summary of Incidence and Mortality Rates.

The combined clinical and laboratory investigation personally conducted resulted in the diagnosis of 22 cases as spirochaetal jaundice out of a total of 51 suspected cases.

Eight of the 22 positive cases were coal miners, i.e., 36.3%. Two of the eight died - a mortality of 25%.

Previous to this investigation, nine other coal miners were considered to have suffered from spirochaetal jaundice: the diagnosis was based solely on clinical symptoms: three died - a mortality of 33%.

In all, seventeen coal miners are recorded as having contracted the disease since 1923, and out of that number five died, making a mortality of 29% amongst coal miners in Scotland.

Fourteen of the twenty two positive cases occurred in persons not associated with coal mines, i.e., 63.3%.

Eleven occurred in young persons of twenty years, and under, six females, five males. No deaths occurred among the eleven young people affected.

Three fatal cases occurred in male adults over thirty five years of age; one was a brewery worker in Edinburgh, one an aluminium worker, and the other a labourer at a piggery in Fife.

The/

The mortality amongst the fourteen persons not associated with mines = 21%.

The following table summarizes the preceding figures:-

Coal miners	17	5died	mortality = 29%.
Other occupations	14	3 died	mortality = 21%
Total, all occupations	31	8 deaths	Mortality for Scotland = 25%

The death rates reported by various Japanese observers are:-

Inada	30.6%
Ogura	40. %
Nishi	48. %

These figures refer to different parts of Japan. Dawson, Hume and Bedson estimated it approximately at 4 - 5% in Belgium, and Martin and Pettit considered that the death rate in France was not more than 5% of cases.

It will thus be seen that the percentage of deaths in Scotland, as a result of spirochaetal jaundice, is much higher than the figures quoted for Belgium and France. The death rate amongst coal miners in Scotland approaches very closely to the Japanese figure stated by Inada.

3. Morbid Anatomy.

The six patients who died were all intensely jaundiced. Two died on the tenth day, one on the eleventh day, two on the twelth day, and one on the fifteenth day from the onset of illness. A complete post-mortem examination was performed in five cases and in one the abdominal contents only were examined.

Heart and Lungs.

A few sub-endothelial haemorrhages were observed in the left ventricle in four cases: in two epicardial haemorrhages were present: in two also the lungs were congested and showed haemorrhagic areas, together with petechial haemorrhages in the trachea and bronchi. In one instance the larynx and trachea were covered with blood clot, the result of haemorrhage from posterior nares, and the oesophagus showed haemorrhagic areas.

Stomach and Intestines.

The stomach was usually "peppered"over with submucous haemorrhages; these were larger and more numerous at the fundus, and in one case a large blood clot was present. In another case extensive and numerous haemorrhages appeared scattered over the mesentery and a few of the mesenteric glands were enlarged and haemorrhagic. The small intestine was commonly found to show small sub-peritoneal haemorrhages which were also evident in the large intestine in one case.

Liver and Bile Passages.

The liver was enlarged in two cases; in one of these, in which chronic hepatitis caused adhesion to the diaphragm, it weighed 2,100 grammes. Varying degrees of bile staining were evident in all, but no obvious macroscopic change was noticed. The common bile duct appeared patent and/

Fig. 3. Submucous haemorrhages, stomach. Case No 16, A.C.

Fig. 4. Subperitoneal haemorrhages in mesentery and haemorrhagic mesenteric gland. Case No 16, A.C.

and allowed the free passage of a probe. The gall-bladder showed no marked distension and usually contained thick, viscid, bile, greenish brown in colour, although in one case the bile was watery and greenish black in colour.

Spleen.

In only one case was the spleen slightly enlarged, soft and diffluent, otherwise it was firm and not enlarged.

Pancreas.

No abnormal features were observed.

Kidneys.

As a rule there was slight enlargement and the capsule was not adherent: small subcapsular, cortical and medullary haemorrhages were present in three cases. On section they were congested and bile stained, and in some instances the colour suggested fatty change: the pelvis was also congested and coagulated blood was found in the calyces in one case.

Bladder.

Apart from greenish bile staining nothing abnormal/

abnormal was noted.

No visible haemorrhages were found in the muscles and with the exception of one case the glands were not enlarged.

The aspect of the macroscopic haemorrhages in the stomach and intestine is illustrated in the accompanying photograph.

Pathological Histology.

The following microscopic descriptions are based on tissues removed at autopsy which was performed at different times in each case, between 24 and 48 hours after death. Portions of the organs were fixed in 10% formalin, and stained with haematein and eosin.

The Liver showed no marked alteration in lobular structure. Varying degrees of cloudy swelling were noted, and in one instance it was very advanced with faintly staining granular cytoplasm and feebly coloured nuclei. Fatty changes, though present, were neither pronounced nor extensive. The sinuses were congested and contained an increased number of polymorphs and lymphocytes; small haemorrhages caused disruption/

disruption of the parenchyma at parts. A feature in most cases was the presence of large accumulations of bile pigment chiefly situated in the liver cells around the hepatic vein (Fig. 5.). Small foci consisting of polymorphs and round cells lying in a matrix of young fibrous tissue were not uncommon and sometimes were related to the smaller bile ducts, (Fig. 9). The portal areas also showed cellular infiltration but not to any marked degree. Liver cell mitosis was also observed but never to the extent described by some observers (Dawson and Hume, Hart and others). Martin and Pettit state that the liver lesions in this disease distinguish themselves by their polymorphism and that between two extreme types of tissue changes, moderate and advanced, transition forms can be recognised. Similar observations have been described by Garnier and Reilly, and Miller. The advanced type of liver lesions is stated to consist of, more or less complete destruction of normal structure with dissociation of liver cells, coagulation necrosis, marked evidence of biliary stasis, and around necrotic areas obvious liver cell mitosis is a feature. This advanced type of liver alteration has also been described by Beitzke, Busch, Dawson and Hume, Garnier and Reilly, Stokes, Ryle and Tytler, and others, but in the few fatal cases examined by the writer such destructive and regenerative liver changes have not been observed.

Fig.5. Liver, Case 21.
Large accumulations of bile pigment.

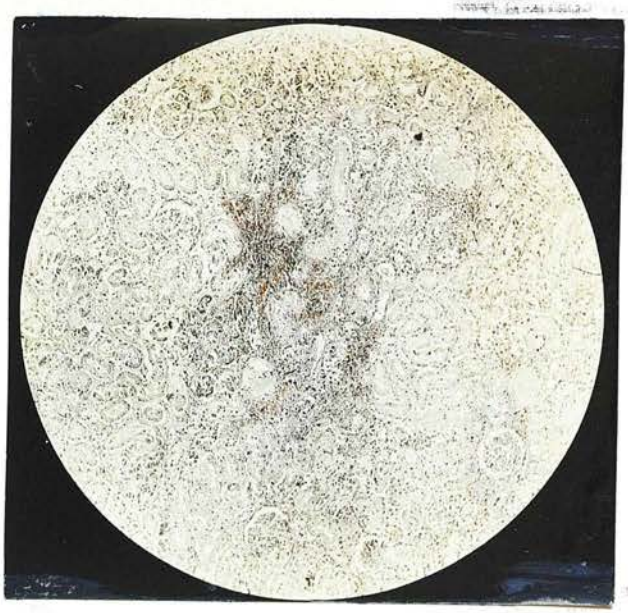


Fig.6. Kidney, Case 16.
Interstitial & tubular haemorrhages in cortex.

Fig.7. Suprarenal, Case 16.
Dissociation of gland structure by haemorrhage.

Fig.8. Spleen, Case 6.
Phagocytosis of red cells, (×) & fragmentation of red cells.

The kidney:- Convoluted tubules showed changes, from that of advanced cloudy swelling to granular disintegration with small local areas of cell necrosis. Haemorrhage into the tubules was evident in some cases, but usually larger haemorrhages were present in the medulla, into the interstitium. Bile pigment was noticeable in the tubular epithelium and in one case was irregularly distributed in localised areas to a marked degree. The glomeruli, apart from show-:ing congestion, were, as a rule, singularly little altered. Small focal accumulations of round cells both in the cortex and medulla were observed. Numerous hyaline casts were present in most cases, and many appeared discoloured by the presence of bile pigment. The collecting tubules showed degeneration and desquamation of the lining epithelium and in many blood casts were seen. These changes accord in the main with those described by other observers (Beitzke, Busch, Miller, etc.), but as in the liver, partial regeneration of the cells was not noticed to any extent.

The suprarenal invariably showed considerable hæmorrhagic change, and in a few cases complete dissociation of gland structure was evident (Fig. 7). The increase in leucocytes and round cells was not so obvious as in the suprarenal of the guinea pig.

The spleen showed atrophy of the Malpighian bodies in all cases, and in several, marked hyaline change in the/

Fig. 9. Cellular infilration around small bile ducts liver. Case No 18 J.M.

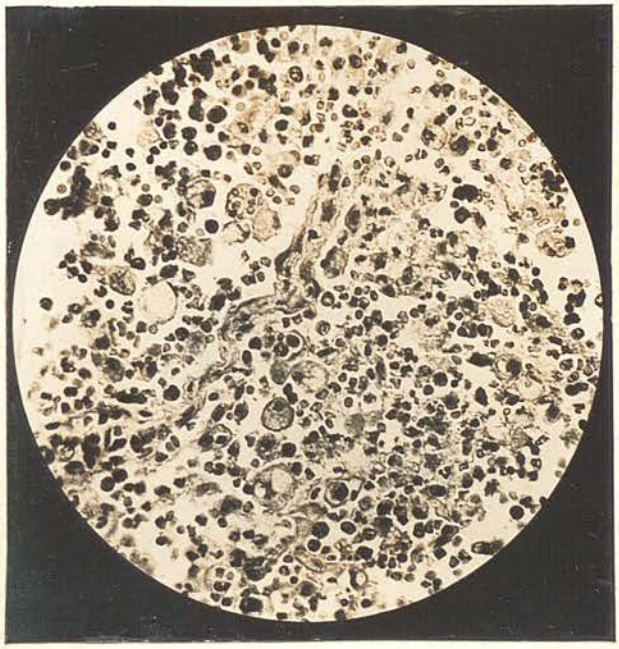


Fig. 10. Phagocytosis of red cells and proliferation of endothelial cells, lymph gland. Case No 16. A.C.

the central arterioles was a feature. Haemorrhagic areas were usually seen and much bile pigment was present. There was considerable increase of fibrous tissue, and although endothelial cell pro-:liferation was noticeable in three cases, it did not occur to such a marked degree as in the spleen of the infected guinea pig (Fig. 28). Similarly with phagocytosis of red cells this occurred, but not to such a pronounced extent as in infected animals.

Fragmentation of red cells was a distinctive feature in one instance (Fig. 8), and small aggregations of altered red cells were scattered all over the pulp. There was usually much haemosiderin pigment present.

The lymph glands were congested and haemorrhagic; endothelial cell proliferation was notable in two cases and phagocytosis of red cells was particularly marked in one instance (Fig. 10).

The lungs, as a rule, were the seat of localised intra-alveolar haemorrhages, marked congestion and oedema. In addition, one case showed small scattered pleural haemorrhages (Fig. 11). Evidence of a terminal broncho-pneumonia was noticed in two instances. Phagocytosis of red cells in the lung has been emphasized by some writers but as described in the guinea pig (p. 59) it was not an obvious feature.

In/

Fig.11. Lung, Case 16.
Pleural haemorrhage.

Fig.12. Heart muscle, Case 16.
Capillary haemorrhages.

Fig.13. Stomach, Case 16.
Small submucous haemorrhages.

Fig.14. Intestine, Case 16.
Subperitoneal haemorrhages.

Fig. 15. Capillary haemorrhage and degeneration of muscle fibre, rectus abdominis. Case No 16 A.C.

Fig. 16. Haemorrhage in gland structure and areolar tissue, pancreas. Case No 16 A.C.

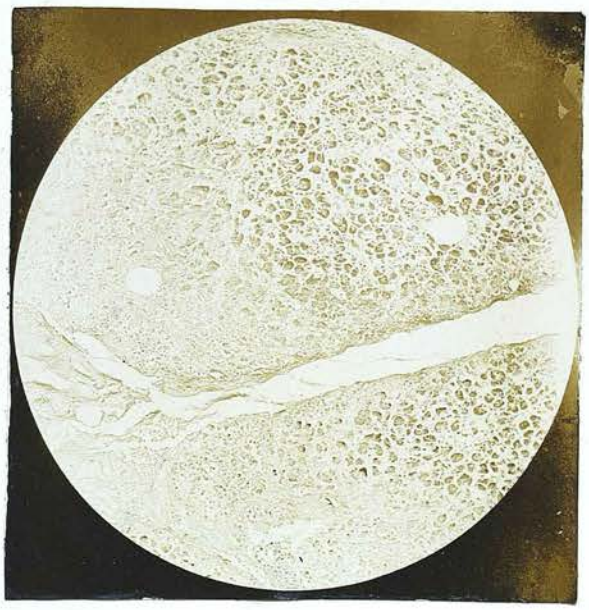


Fig. 17. Necrosis of gland tissue, pancreas. Case 16 A.C.

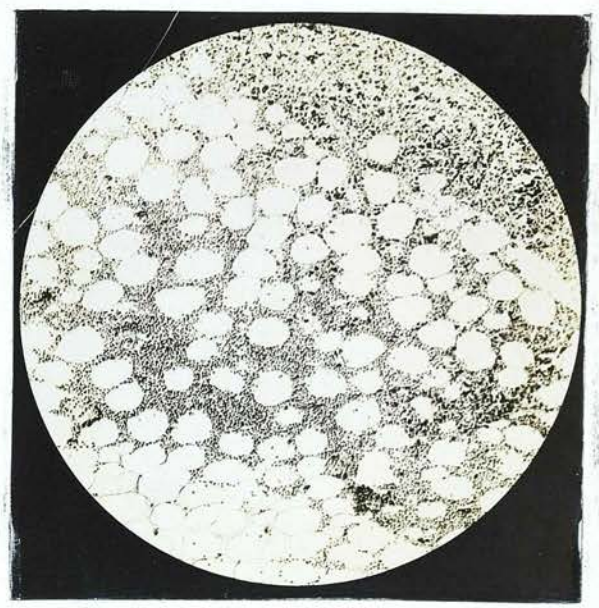


Fig. 18. Periglandular haemorrhage, mesenteric gland. Case No16 A.C.

In the other organs and tissues the following changes were noted :- The heart muscle and stomach wall showed small capillary haemorrhages, and the small intestine, sub-peritoneal and inter-muscular haemorrhages. The Peyer's patches were not noticeably altered as in the experimental animal. In the pancreas small haemorrhages were present in the gland structure (Fig. 16), in one instance along with areas of marked necrosis (Fig. 17). In this same case the muscle fibres of the rectus abdominis showed degeneration and capillary haemorrhages were present (Fig. 15). The bladder wall showed congestion of the blood vessels but no haemorrhages.

The microscopic changes in the tissues of both human and guinea pig forms of the disease were found to be strikingly similar, and varied only in degree (vide p. 56).

Distribution of the spirochæte in the human tissues.

In the five fatal cases tabulated (p. 16), spirochaetes were demonstrated microscopically in sections of certain tissues, prepared by a modified Levaditi method (p. 74). Portions only of the liver in a sixth case were available for examination, but no spirochaetes were found. The patient died previous to this investigation and the case has been intentionally omitted from the table (p. 16).

Pieces/

Fig.19. Case 16. Liver.

Fig.20. Case 16. Kidney.

Fig.21. Case 18. Kidney.

Fig.22. Case 16. Lung.

Fig.23. Case 16. Heart.

Figs.19-23 Micro-photographs of sections of tissues prepared by modified Levaditi method, to show spirochaetes in human organs.

Pieces of practically all the organs and tissues of Case 16 were sectioned and examined. This patient died on the 12th day of illness, and although spiro-:chaetes were found in the liver, kidney, lung and heart muscle (Figs.19,20, 22,23), they were by no means numerous. No spirochaetes were found in any of the other structures. In the tissues of the four other patients who died between the 11th and 15th day from the onset of illness, a few spirochaetes were found, only in kidney sections in three instances, and in the liver in one case. The appearance of the spiro-:chaete, as seen in sections of tissues prepared by the Levaditi method or its modifications, is con-:sidered later (p.74), but it may be mentioned here that the distinctive morphology in section is entirely obscured.

Kaneko and Okuda reported on the distribution of the spirochaete in the organs and tissues of sub-:jects who died between the 6th and 10th day of illness. They described them as most numerous in the liver and kidney, both in extracellular and intracellular situations. Other organs and tissues showed spirochaetes in decreasing numbers according to the following order :- The suprarenals, heart muscle, intestinal wall, appendix, pancreas, prostate, lungs, spleen, lymph glands, skeletal muscle (particularly gastrocnemius) and bladder wall. It was also stated that the spirochaetes gradually disappeared/

disappeared from the tissues and organs, with the exception of the kidney, in the later stages of the disease and during convalescence.

III. EXPERIMENTAL INVESTIGATION.

1. Historical.

The causal organism of the disease was discovered by the Japanese workers Inada and Ido, who, with their collaborators Hoki, Kaneko, Ito, Wani, and Matsuzaki, investigated epidemics of febrile jaundice in Japan. The condition has been prevalent in epidemic and endemic form in certain parts of Japan, and in some regions was known by the name odan-eki (icteric pestilence). It was considered to be the Japanese equivalent of the condition designated Weil's disease or infectious jaundice in Europe.

In November 1914 Inada and Ido observed spirochaetes in the liver of a guinea pig inoculated with the blood of a patient suffering from infectious jaundice. They confirmed this observation some months later by repeating the animal experiments, which resulted in the development of jaundice and haemorrhages in the infected guinea pigs, and spirochaetes were demonstrated in the liver and blood.

It/

It was also proved that the infection could be transmitted from animal to animal. The initial results of their important research were first published in February 1915 in a Japanese Journal, and in 1916 an English version appeared with a description of further work on the subject. From the experimental results they concluded that the spirochaete was derived from the blood of the infected individuals, and this they subsequently proved by demonstrating a spirochaete of similar morphology in the blood of six patients. They also detected it in sections of the intestinal wall and suprarenal of two out of eleven persons who died between the 8th and 14th day of illness, and, in two later patients who died on the 6th day, numerous spirochaetes were found in the liver.

The elimination of the spirochaete in the urine was stated to occur from the 13th to the 40th day from the onset of illness. Moreover, in the blood of five recovered patients they demonstrated the presence of specific protective, curative, and spirochaeticidal substances by means of animal experiments. These properties were not demonstrable in the serum of persons with jaundice due to other causes. In this manner they established the rôle and specificity of the/

the micro-organism named by them spirochaeta icterohaemorrhagiae, and this resulted in distinguishing from the other types of jaundice often confused with this spirochaetal form, a nosological entity, to which the Japanese authors applied the name of spirochaetosis icterohaemorrhagica.

The English description (1916) of the Japanese work has formed the basis on which much further research has been accomplished throughout the world.

Although writers in most countries accord priority for the discovery of the micro-organism of the disease to Inada and Ido, nevertheless certain German observers already mentioned dispute the claim. The disputants, Hübener and Reiter, and Uhlenhuth and Fromme, did not record their discovery until 1916; they failed to recognise and describe the exact morphology of the spirochaete, which they found in animals infected with the blood of patients suffering from Weil's disease. Reference is made by the French writers, Martin and Pettit, to a German "tribunal" which met to decide the rival claims of the authors mentioned; according to the French workers, the only omission at the enquiry was the names of Inada and Ido! The work of the Japanese observers however, was/

was certainly confirmed by these German investigators and by Beitzke in 1916. Experimental inoculation into guinea pigs of blood from suspected cases of infectious jaundice was the means used by Stokes, Ryle, and Tytler, and Dawson, Hume and Bedson, in establishing the existence of spirochaetal jaundice amongst British soldiers in Belgium in 1916. Later in the same year Martin and Pettit, Costa and Troissier, Garnier and Reilly, and others established the prevalence of the disease amongst French soldiers, and Monti, and Sisto, proved by animal experiments its existence on the Italian front. Since the end of the War the occurrence of the disease in many other countries, e.g. England, United States, Cuba, South America, Sweden, Sumatra and Scotland, has been similarly established by the authors already quoted (p.10), and many writers have substantiated the work of the Japanese observers.

2. Experimental Inoculation.

Numerous species of animals have been inoculated with spirochaeta icterohaemorrhagiae by several workers to determine the relative degree of susceptibility/

susceptibility to infection. This enables the following grouping to be made:-

(a) Animals refractory to infection.

(b) Animals harbouring the spirochaete as a commensal without affecting the host.

(c) Animals susceptible to infection and to the pathogenic effects of the organism.

(a) This group includes the cat, pig, sheep, rabbit, white rat and mouse, hedgehog, hen, pigeon and frog. Martin and Pettit state that inoculation of large doses of the spirochaete into these animals generally fails to produce any marked signs of infection; death is exceptional, and in those that succumb after inoculation death is not always attributable to spirochaetal infection. However, in the case of the rabbit, white rat and mouse, this is not constantly so, as several workers, viz. Kaneko, Ido, Hoki and Wani, Uhlenhuth and Fromme, Monti, and the writer have observed that jaundice may occur in these animals following the inoculation of the virus. According to Dawson, Hume and Bedson the monkey also is more or less immune.

(b) This class includes the wild rat and field mouse/

mouse which are fully dealt with in Section IV.(p. 88). Both act as natural carriers and disseminators of the organism which has no pathogenic effect on either host. From the point of view of aetiology the dog may also by included in this category, because it has been shown by Uhlenhuth and Fromme (1919), and Okell, Dalling and Pugh (1924), that the infection may occur naturally in this animal, as in the wild rat and field mouse, but unlike these rodents, the dog may succumb to the pathogenic effects of the organism.

(c) The third group is represented pre-eminently by the guinea pig which is extremely susceptible to the pathogenic action of spirochaeta icterohaemorrhagiae. According to Courmont and Durand (1917), Monti (1917), and Okell, Dalling and Pugh (1924), puppies experimentally infected also proved susceptible to the disease.

The human species corresponds to the guinea pig in being frankly susceptible to the spirochaetal infection.

The Disease in the Guinea Pig.

In the following research the writer has used the animal of choice, the guinea pig, both for experimental inoculation and in making a comparative study of the disease.

Technique of Inoculation.

The materials that may be employed for experimental inoculation in investigating the disease include:-

a. Infective blood, urine, and cerebro-spinal fluid from human cases.
b. Kidney and urine of animal carriers.
c. Tissues and body fluids of infected guinea pigs.
d. Virulent laboratory cultures of the organism.

The infective material used in the following work was obtained from the last three sources, (b, c, and d.)

Method of preparation and dose of inoculum.

Portions of infected animal tissues e.g. kidney, liver, and suprarenal, were cut into small pieces in a mortar, ground up with sterile sand, and 10c.c. of normal saline/

saline were added. Aseptic precautions were observed in the process. The sand was allowed to sediment and the supernatant fluid containing the tissue in a fine state of subdivision was used; a dose of 1 - 2 cc. was inoculated. Body fluids and virulent cultures were injected in similar amounts.

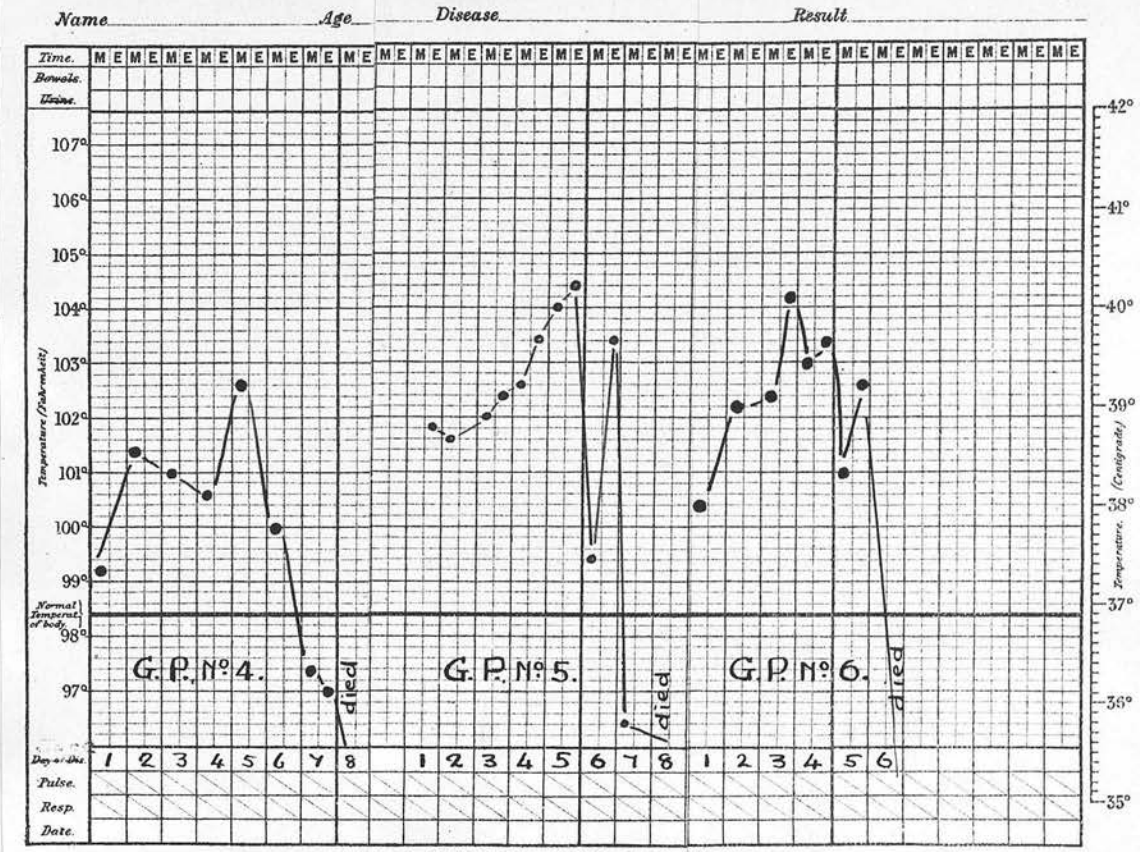
Mode of Inoculation.

Intraperitoneal inoculation was the method found most suitable for the object in view, although certain writers mention the risk of peritonitis, following the injection; in my experiments however, this complication was not encountered. Other methods of inoculation were tested, but are considered in relation to modes of spread in Section IV, (p. 121).

Signs of the Disease in the Guinea Pig during Life.

The characteristic and most constant features were found to be pyrexia and jaundice. Haemorrhages are included among the cardinal signs by Dawson, Hume and Bedson, Martin and Pettit, and others, but of over fifty guinea pigs examined, only six showed external haemorrhages. Other notable features were anaemia and conjunctival congestion. Spirochaetes usually appeared in the blood about the fourth day of illness.

The/



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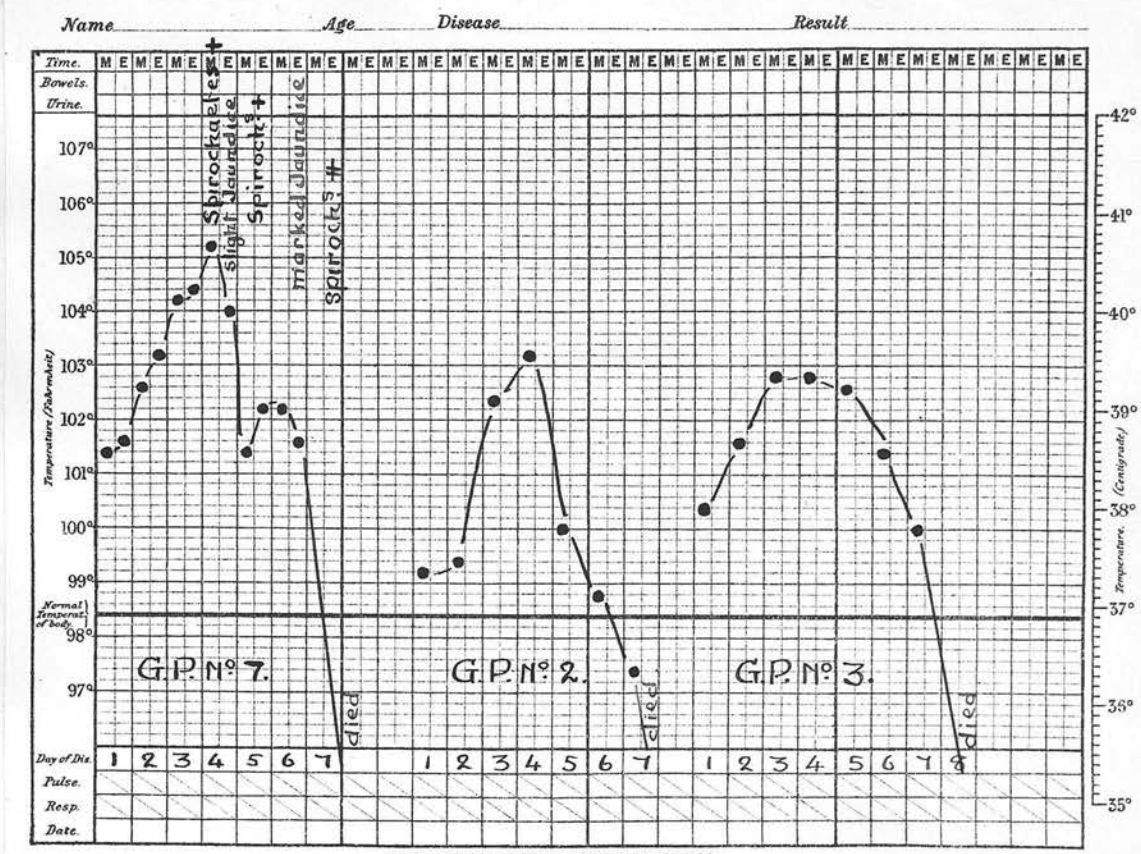
RECORDS OF TEMPERATURE IN INFECTED GUINEA PIGS.



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The Fever invariably manifested itself on the first day after inoculation, by a rise in rectal temperature varying from 0.5° to even 2°F in some cases. The average normal temperature of the guinea pig is in the neighbourhood of 100°F and in the accompanying charts a characteristic feature is seen in the rapid rise of temperature to its acme within a few days. It then falls to normal and finally becomes subnormal during which period death occurs, usually between the fifth and twelth day of illness. The highest temperature noted was 105.3°F, and in two moribund animals the rectal temperature was considerably below 95°F; these died during the manipulative process of taking the temperature.

Jaundice was first appreciable when the temperature began to fall, usually about the fourth or fifth day after inoculation. In light coloured animals the development of the icterus was readily observed by the gradual change in colour of the ears, nose and pads of the feet, and in others it was evident on the conjunctivae and mucous surfaces. The jaundice is characterized by its extreme intensity, the tint being exactly simulated by treating the skin of the guinea pig with strong picric acid solution; it is concomitant with the fall of temperature and becomes increasingly more pronounced until the day of death.

Choluria/

Choluria is usually an accompaniment, and albumin, tube-casts, red blood corpuscles and spirochaetes were frequently found in the urine. External haemorrhage is stated to be frequent though not constant in occurrence (Martin and Pettit), and may arise from the nose, intestine and genital organs. This symptom was observed in only six guinea pigs amongst over fifty examined; three showed blood effusions at the anus, two at the nose, and one at the genitals.

Symptoms common to other infections were also noted e.g. anorexia, loss of weight, weakness, drowsiness and inaction, and on the day of death the infected animals became palpably cold.

The symptoms described are, on the whole, typical of spirochaetal jaundice in the guineapig, but the following abnormal forms were met with in the series of animals examined. The guinea pigs are referred to in the tabulated protocols (pp.93-100) under the following numbers: - 1, 21, 23(2nd G.P), 35, 36, 72, 78, 79, 83, 84, 87, 89, 91, 96,100&132.These sixteen animals were inoculated with infected kidney tissue from sixteen wild rats respectively, in the course of an enquiry, described later, into animal carriers of the disease. None of them showed any evidence of jaundice, and with the exception of No.96,[1] signs of acute illness were absent. Twelve remained alive and apparently well for several weeks beyond the usual fatal period (vide protocol). In one animal, No.100, which lived thirty five days after inoculation, spirochaetes were found in/.

1. Temperature rose to 103° F. on 4th day after inoculation.

in the urine on the twenty fifth day when no signs of illness were obvious.

The diagnosis of spirochaetal jaundice was readily made during life, on the evidence of the above mentioned typical signs alone, in over fifty of the inoculated guineapigs; but this was supplemented in each case at death - usually 5-12 days after inoculation - by bacteriological confirmation. This was done by demonstrating under the dark-ground microscope, the specific spirochaete in the organs or body fluids of the infected animals. The procedure adopted is described later (p. 66).

The diagnosis of the disease in guinea pigs that behave abnormally after inoculation of infected material, as in the sixteen animals mentioned, is sometimes a difficult matter, and usually depends on the post-mortem findings which are considered in detail (p. 50).

The protocols of the experiments on which most of the foregoing observations are based, relates also to later work with which it will be found associated in Section IV(pp.93-100).

Blood Changes in the Guinea Pig.

Observations were made on twelve animals: eight (Group A), were inoculated with 2c.c. of liver emulsion from recently dead infected guinea pigs, and four (Group B), with 1c.c. of a virulent laboratory culture, seventeen days old, of the spirochaete grown in a medium containing defibrinated rabbit blood, agar and normal saline (vide p. 78). Blood examinations were performed each morning until the infected animals died.

The average blood count in the twelve animals was found to be as follows:- red cells 6,336,600 per c.mm., white cells 7,336 per c.mm.. In these animals no wide variation in the number of cells was noted: thus the highest count of red cells was 8,200,000 per c.mm., the lowest 5,840,000 per c.mm.; in the case of white cells the range was from 15,312 per c.mm. to 6,506 per c.mm.. The average haemoglobin percentage was 88.7, the range being from 99 to 82 per cent. The results of the blood examination in four of these animals may be disregarded: of two in Group A, one died of pseudo-tuberculosis, and one was an atypical case of the infection with absence of jaundice (vide p. 55,c): two in Group B inoculated with what was originally a virulent culture failed to/

to become infected, due, either to attenuation in virulence of the culture or, to natural immunity in these individual animals.

The following figures indicate the results obtained in six of the animals, the corresponding teperature charts of which are shown (p. 37).

G.P. No.4 inoculated with infected liver emulsion.

Date.	Red cells. per c.mm..	Hb%	White cells. per c.mm.
10.6.25 before inocn.	8,000,000	99	15,312
11.6.25 after inocn.	7,520,000	92	11,250
12.6.25 " "	7,840,000	94	10,000
13.6.25 " "	8,000,000	90	13,750
14.6.25 " "	8,100,000	90	13,750
15.6.25 " "	6,560,000	88	14,375
16.6.25 " "	4,800,000	60	14,843
17.6.25 found dead in the morning.			

G.P. No.5 inoculated with infected liver emulsion.

Date.	Red cells. per c.mm..	Hb%	White cells. per c.mm.
19.6.25 before inocn.	6,900,000	98	8,000
20.6.25 after inocn.	6,000,000	98	6,562
22.6.25 " "	6,080,000	84	8,125
23.6.25 " "	5,600,000	84	9,062
24.6.25 " "	6,960,000	84	7,185
25.6.25 " "	5,600,000	88	10,000
26.6.25 " "	4,640,000	66	15,312
27.6.25 " "	5,600,000*	60	6,250

* The last examination was made from blood obtained by heart puncture just before the animal died.

G.P. No.6 inoculated with infected liver emulsion.

Date.	Red cells. per c.mm..	Hb%	White cells. per c.mm..
27.6.25 before inocn.	8,200,000	98	8,000
28.6.25 after inocn.	7,600,000	96	7,816
29.6.25 " "	6,400,000	94	5,312
30.6.25 " "	6,640,000	99	9,062
1 .7.25 " "	6,140,000	92	8,437
2 .7.25 " "	4,240,000	64	6,563

G.P. No.7 inoculated with infected liver emulsion.

Date.	Red cells. per c.mm..	Hb%	White cells. per c.mm.
22.5.25 before inocn.	6,400,000	82	6,506
23.5.25 after inocn.	6,900,000	80	5,616
24.5.25 " "	6,400,000	84	6,800
25.5.25 " "	6,240,000	78	8,000
26.5.25 " "	4,710,000	64	10,800
27.5.25 " "	3,200,000	60	7,520
28.5.25 " "	3,010,000	40	6,300

G.P. No.2 inoclulated with virulent laboratory culture R. 40.

Date.	Red cells. per c.mm..	Hb%	White cells. per c.mm.
29.5.25 before inocn.	6,000,000	86	7,180
30.5.25 after inocn..	5,600,000	80	5,313
31.5.25 " "	5,600,000	84	10,937
1.6.25 " "	5,320,000	80	9,000
2 .6.25 " "	5,400,000	80	10,250
3. 6.25 " "	4,810,000	76	8,000
4. 6.25 " "	4,010,000	68	5,334

G.P. No.3 inoculated with virulent laboratory culture R.40.

Date.	Red cells per c.mm.	Hb%	White cells per c.mm.
4.6.25 before inocn.	5,840,000	92	10,937
5.6.25 after inocn.	5,920,000	90	8,151
6.6.25 " "	6,240,000	86	8,437
7.6.25 " "	5,600,000	89	5,976
8.6.25 " "	5,520,000	82	5,000
9.6.25 " "	4,480,000	72	7,760
10.6.25 " "	3,040,000	44	15,000
11.6.25 " "	2,800,000	42	3,750

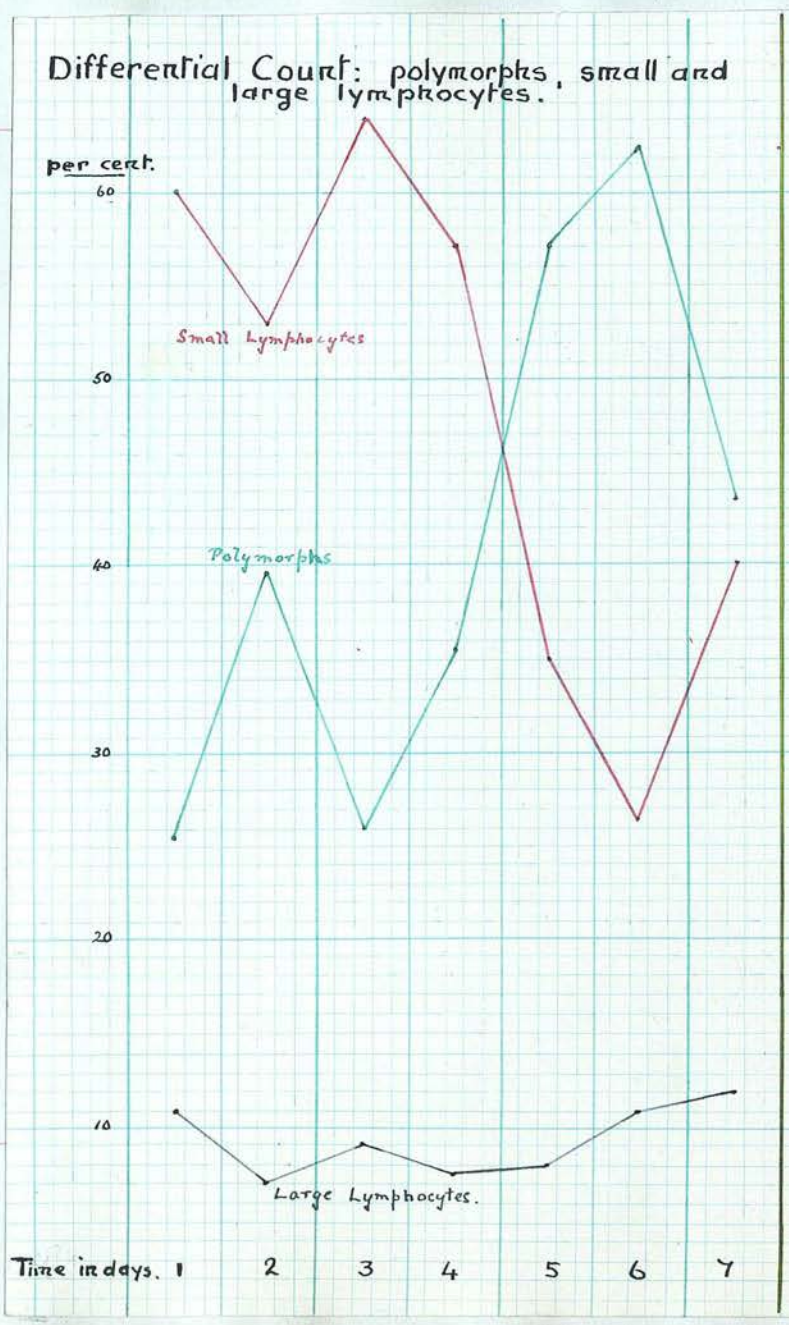
The last count was made from blood obtained by heart puncture when the animal was moribund.

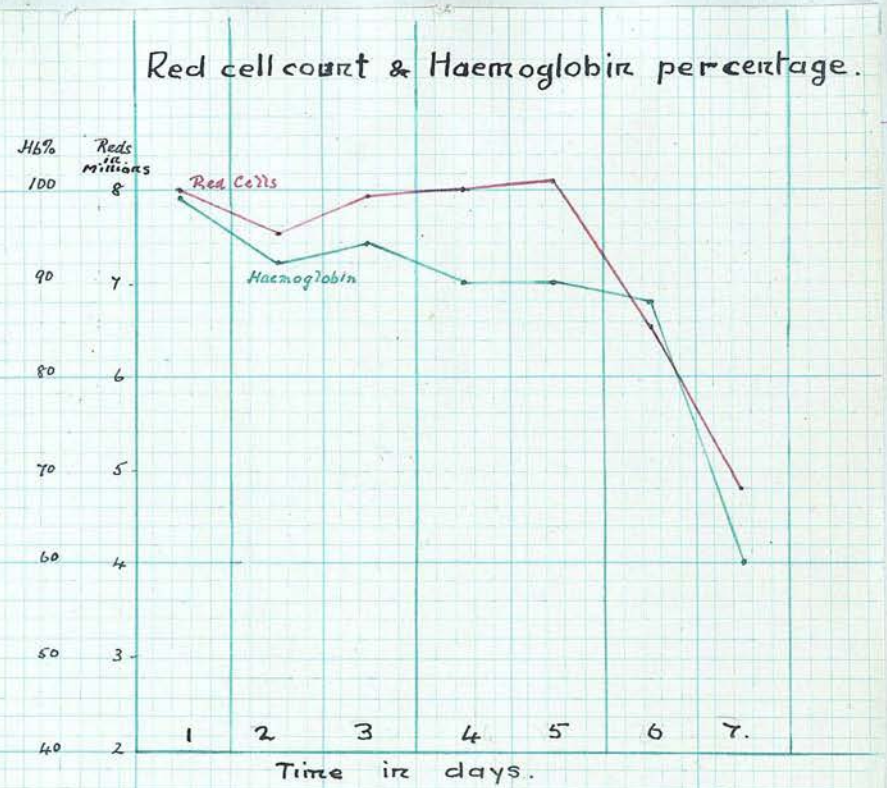
The differential count of leucocytes was also done, and although the percentage of eosinophiles and basophiles, etc., was noted the figures were not charted, as no marked fluctuation in their number occurred. The results of the differential count (small and large lymphocytes, and polymorphs), the total white, and the red cell count, along with the haemoglobin estimation, are displayed in graph form.

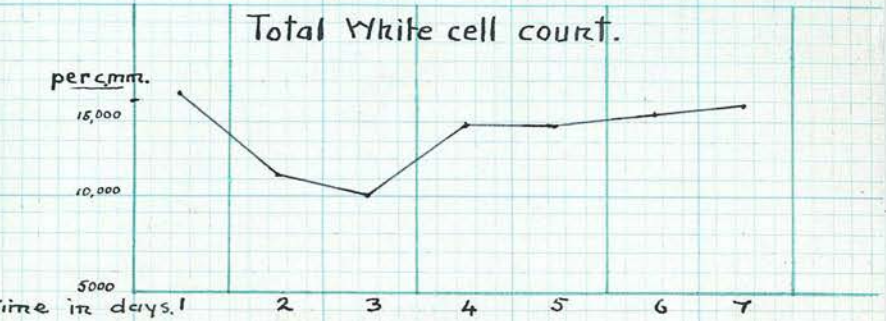
This study of the blood changes in the guinea pig has revealed certain interesting results. A notably consistent phenomenon is shown in the remark-:able graph indicating the differential counts. From the practical point of view, if this obtains in human infection a further aid to diagnosis might be found in determining the presence or absence of a relative small lymphocytosis in suspected cases of the disease. This occurred in the animals examined within the first few days of infection, which period in/

in human cases is of great importance from the aspect of anti-serum administration. The observ-:ation has just been made; no reference to it has been found in the literature, and no human cases have been reported to me since it was noted. Con-:sequently it has not been possible to determine if a similar lymphocytosis also occurs in human cases in the early stages of infection.

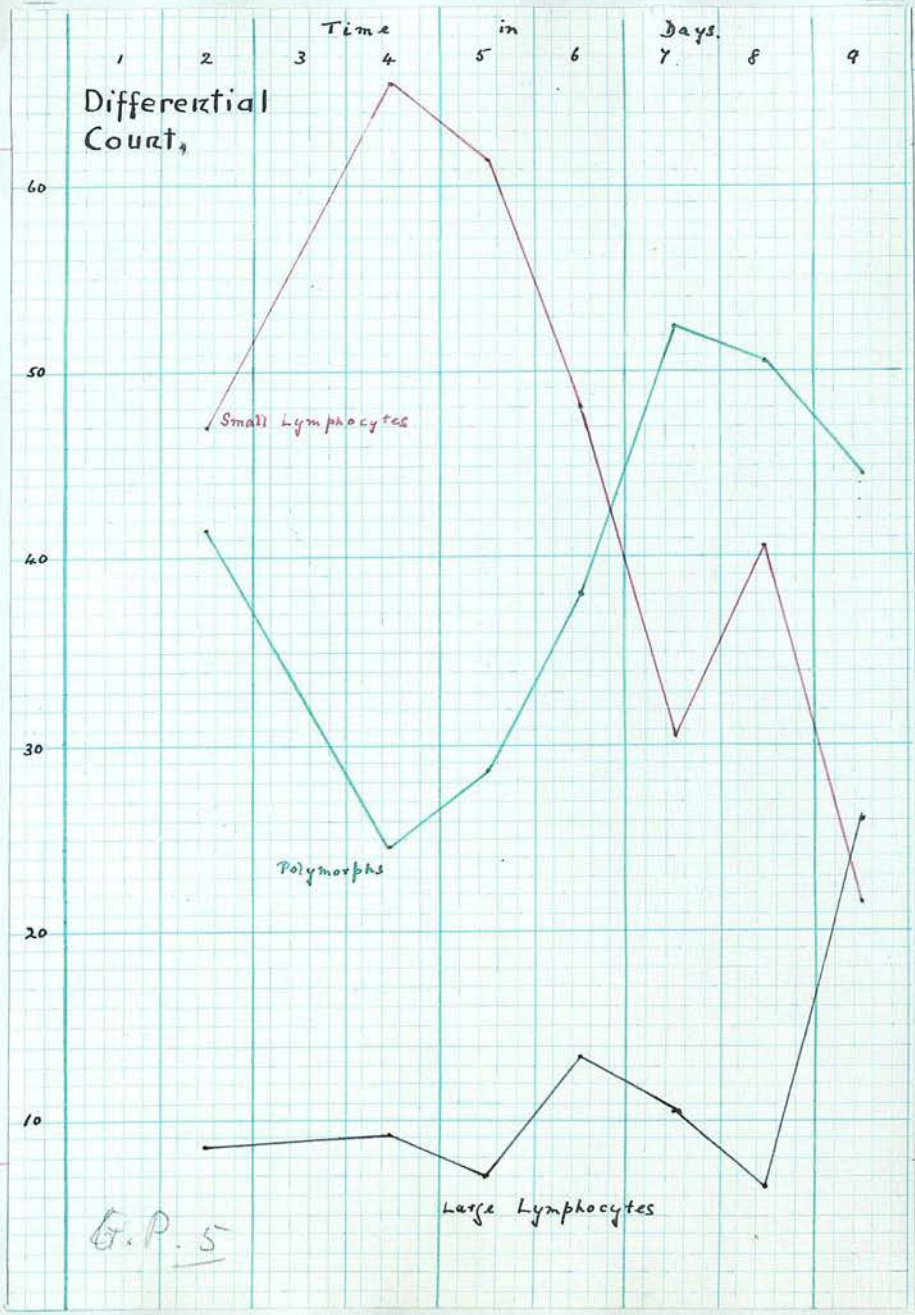
The blood changes in relation to the occurrence of jaundice are discussed in a later section (p. 62).

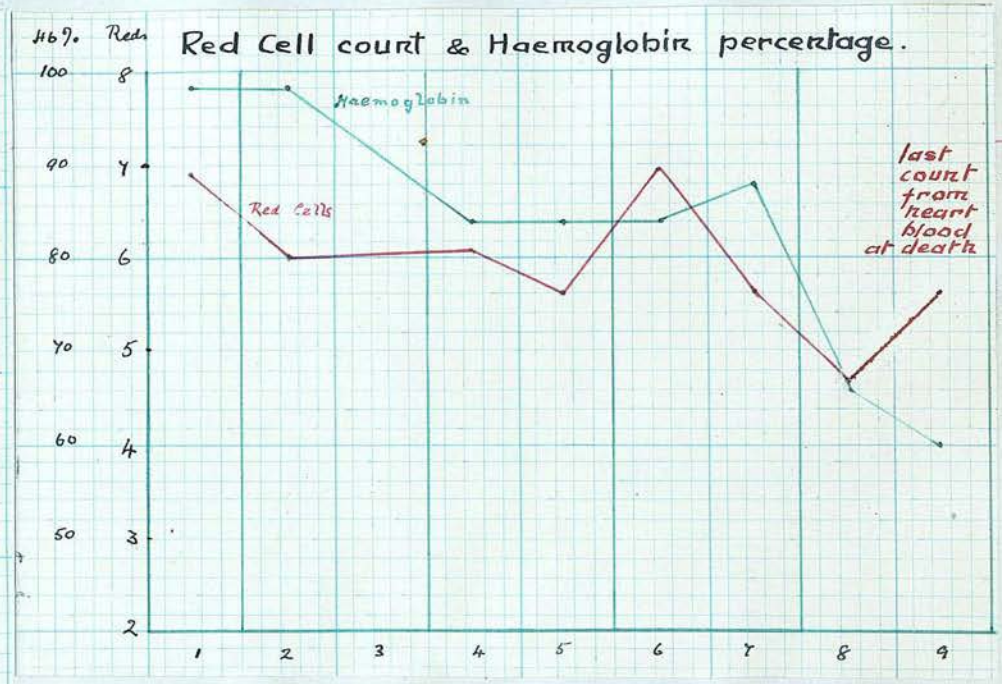


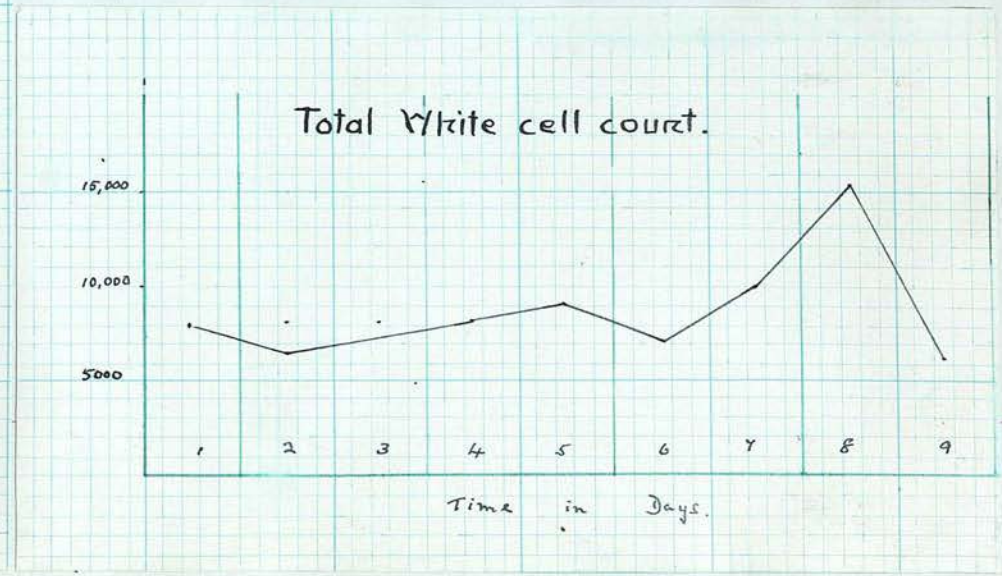




RECORD OF BLOOD EXAMINATION: GUINEA PIG No. 4.







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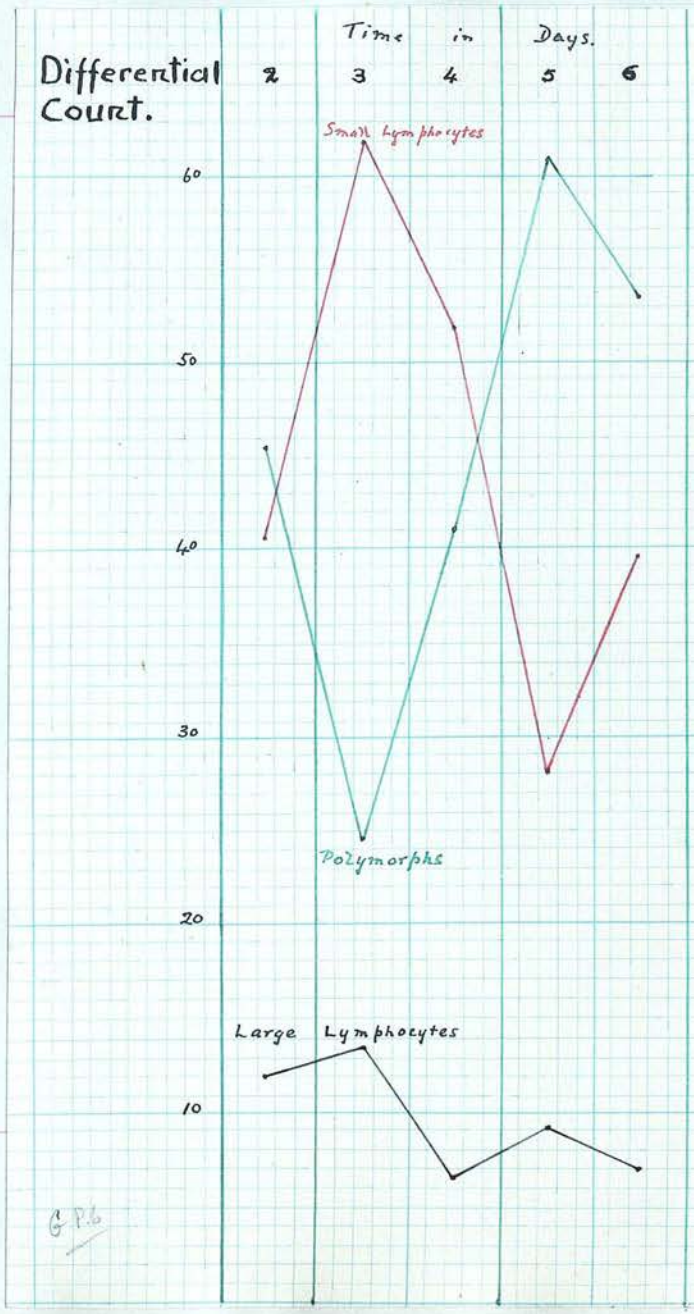
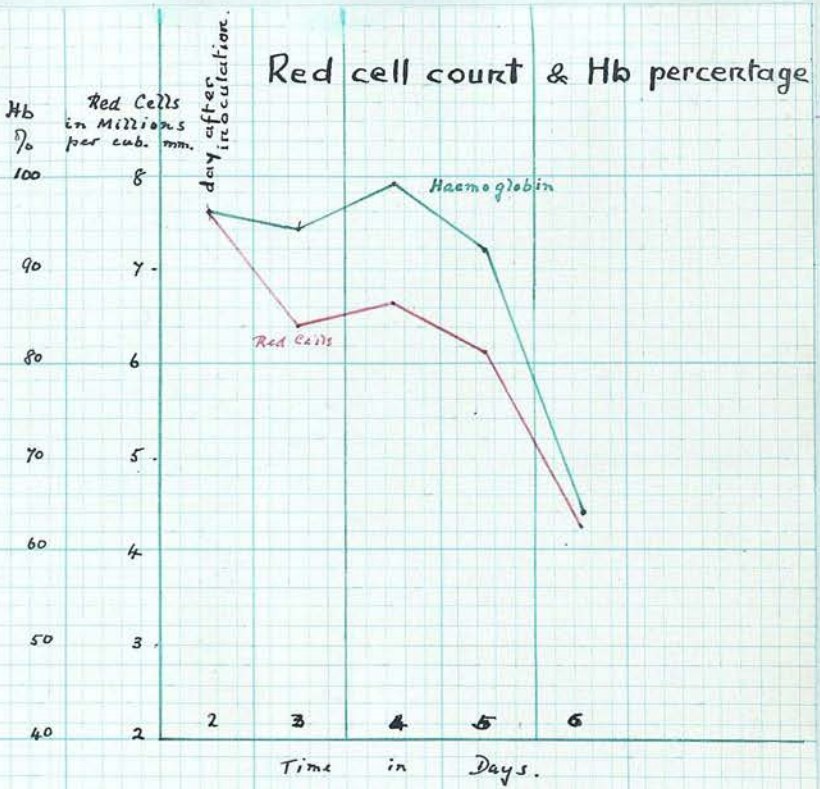
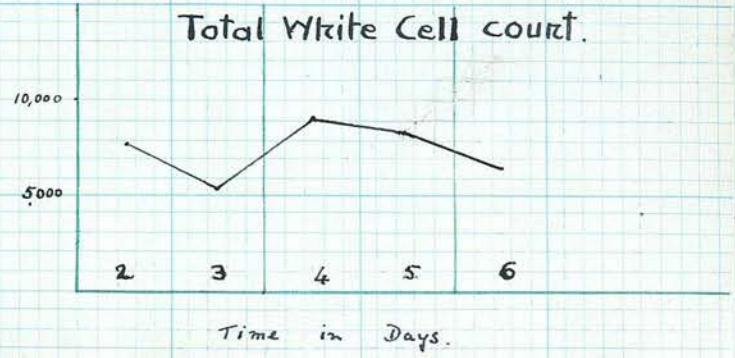
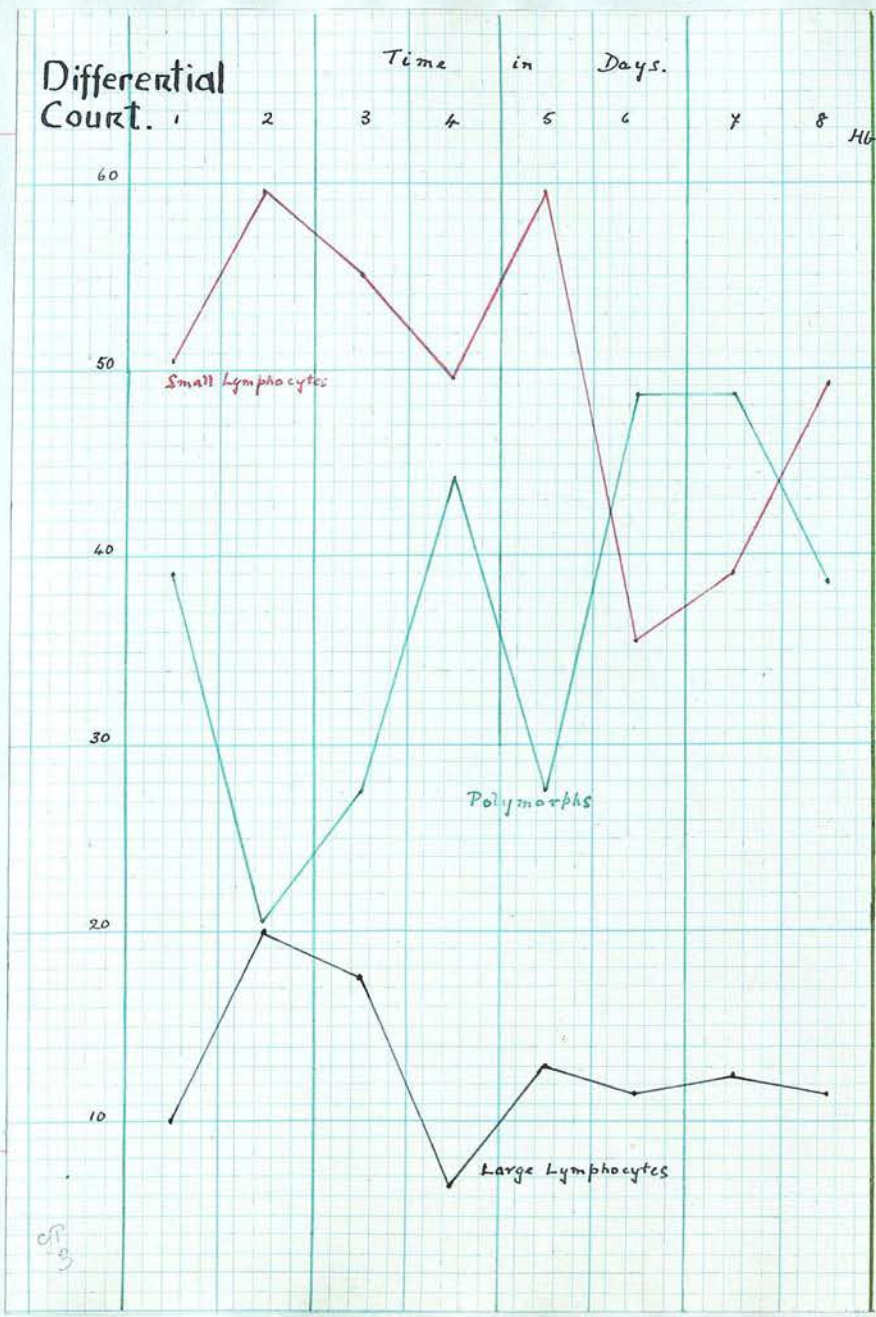
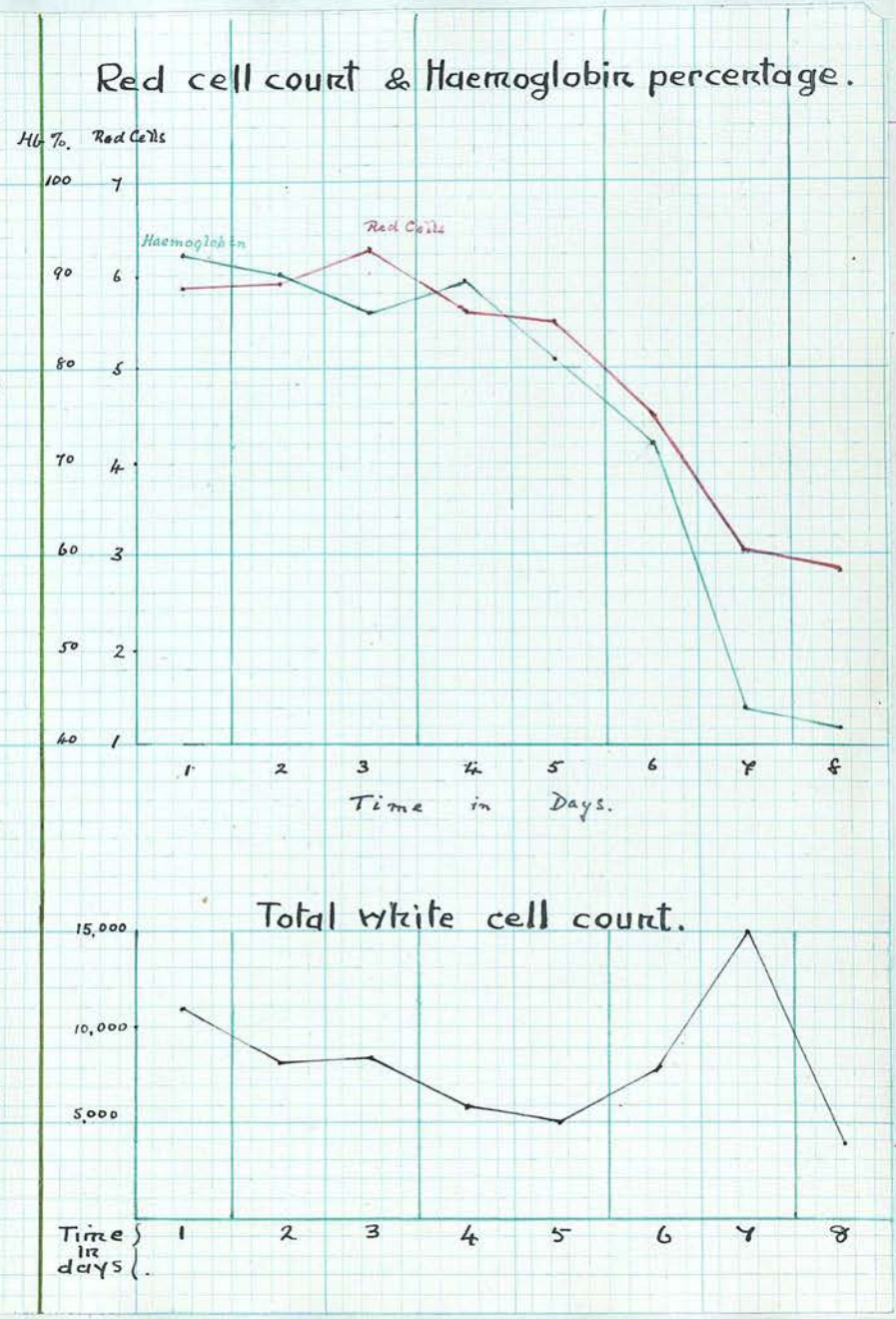






RECORD OF BLOOD EXAMINATION: GUINEA PIG No. 6.





RECORD OF BLOOD EXAMINATION: GUINEA PIG No. 3.

Pathological Changes in the Guinea pig.

Morbid Anatomy.

The following description indicates the salient features found in experimentally infected guinea pigs at autopsy; it is based on the examination of over fifty animals which showed typical evidence of spirochaetal jaundice at death. The majority (vide protocols pp.93-100), were inoculated with an emulsion of infected kidney tissue from wild rats, and a varying number of the minority received an injection of infected material derived from two other sources viz., liver of dead infected guinea pig, and virulent laboratory culture of spirochaeta icterohaemorrhagiae. The pathological picture was more or less uniform in 75% of the cases, and the remaining 25% presented atypical features (vide p. 53).

The animals were usually emaciated, and in those of light colour, jaundice was strikingly obvious in the integuments which presented a deep yellow colouration. In some cases haemorrhages were present at the nose, anus or genitals.

On/

On reflection of the skin, the subcutaneous layer showed a distinctive yellow tinge with numerous haemorrhages of varying size, scattered over the reflected skin surface. In the regions of the axilla and groin extensive haemorrhage was a feature, and the lymph glands were occasionally enlarged and haemorrhagic. The muscles, particularly of the lower jaw and thigh, showed blood effusions over the surface.

In addition to the above features several animals inoculated with a virulent laboratory culture showed very marked oedema and extensive haemorrhages all over the abdominal wall.

On opening the body cavities a slight yellowish colouration of the bladder, intestines, stomach and sternum was noticeable.

The Lungs invariably presented a characteristic appearance ; deep red haemorrhagic areas, irregular both in size and shape, and sharply defined from the surrounding lung tissue, gave to the organ a distinctive spotted appearance, aptly compared to that of the wings of a species of mottled butterfly. This lung lesion is considered by Inada to be a most important diagnostic sign of the disease in the guinea pig, (vide table p.129).

The/

The Heart frequently showed epicardial petechiae.

The Liver, as a rule, apart from congestion, showed no constant or marked naked eye change. In some cases it was enlarged, and in one particular animal it was markedly hypertrophied, and the surface showed distinct variations in colour indicative of fatty change, congestion, and haemorrhage. Two principal types of liver lesions are described by Martin and Pettit viz., fatty, and necrotic changes; these however were not prominent macroscopic lesions in the animals autopsied for the present study.

The Spleen was always more or less enlarged and appeared congested and haemorrhagic.

The Kidneys in some cases showed a slightly greenish yellow pallor, suggestive of bile staining and fatty change, and others appeared intensely congested and haemorrhagic, lying in a capsule suffused with blood. Extravasations of blood from the kidney were of frequent occurrence, and often extended in the retroperitoneal tissue down to the pelvis.

The Suprarenals were frequently enlarged and haemorrhagic.

The Stomach beyond an icteric tinge usually showed nothing/

nothing of note, but in several animals haemorrhages were present on the mucous and peritoneal surfaces.

The Intestine, particularly the large, was frequently the seat of intense congestion and haemorrhage, and a few discrete haemorrhages were occasionally found in the ileum.

The Genital Organs in a few animals showed haemorrhages, which were found situated in the bladder and epididymis of the male, and in the uterus of the female.

The general characters of these pathological lesions are depicted in the accompanying coloured photograph; although this appearance was invariably the rule, still exceptions were noted, and refer to the same sixteen animals which also failed to show the characteristic signs and symptoms of infection during life (p. 39). These animals may be arranged into three groups; the numbers correspond to those in the protocols (pp. 93-100).

A. Immune animals, six in number: of these five were inoculated with infected kidney of wild rat (Nos. 1, 21, 23 (2nd G.P), 83 and 132), and the sixth with liver containing abundant spirochaetes from a guinea pig originally infected from rat No./

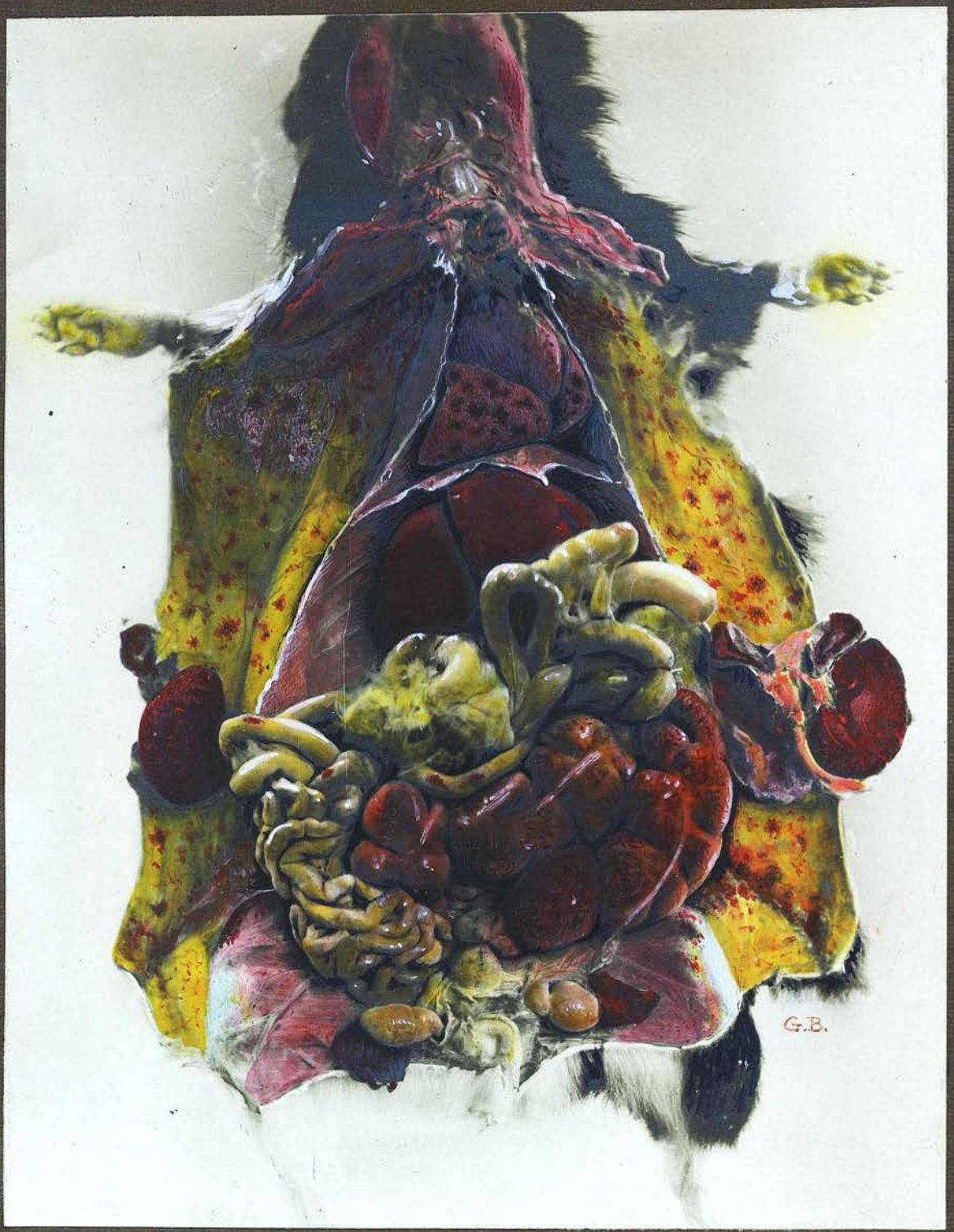


Fig. 24. Guinea pig, died on 10th. day after inoculation of infected kidney tissue from local wild rat.

No.34. All lived well beyond the usual fatal period, none showed jaundice or other signs of infection after inoculation, and no spirochaetes were ever found in the blood or urine during life. Four of the animals died between the 30th and 117th day after inoculation; at autopsy there was no evidence of spirochaetal jaundice and no spirochaetes were found in the body fluids, tissues, or organs. Two animals remained alive and well indefinitely, (Nos. 132 and 23 (2nd G.P), the latter of which received a later inoculation with heavily infected guinea pig liver: it remained insusceptible to infection.

B. Animals that survived for a considerable period, 23 - 110 days, after inoculation, which never at any stage showed jaundice, and in which the only naked eye evidence of infection at death was the presence of old contracted lung haemorrhages. These were eight in number, (Nos. 35, 72, 78, 79, 84, 91, and 100). Spirochaetes, though scanty, were found in the kidney (No. 79) and in the urine/

urine (No.100). These findings are of much significance in relation to the results of animal inoculation with infected human urine (vide table p.129).

C. Animals that died within the usual fatal period, 5 - 12 days, after inoculation, three in number. Two of these (Nos. 36 and 87) failed to exhibit the complete pathological picture of the disease; typical lung haemorrhages were the only visible signs of infection. In one, No 96, slight axillary and subcapsular kidney haemorrhages were the only lesions. In the three animals there was absolutely no jaundice. In two no spirochaetes were found in the body fluids or organs, and in one (No.96), they were present in small number only in the kidney.

Pathological Histology.

The Liver presented a atate of general cloudy swelling, which varied in different animals from a moderate degree of parenchymatous change to advanced granular disintegration with destruction of lobular formation. Congestion was invariably present, and not infrequently intense, in the portal and hepatic veins, and sinuses. Moreover the sinuses contained an excess of leucocytes, polymorphs chiefly and lymphocytes, but contrary to the experience of Monti, phagocytosis of red cells was not observed to any extent in the liver. Disruption of the parenchyma (Fig. 25), occurred as a result of intercellular haemorrhage and oedema, at times in relation to the portal areas. The portal tract frequently showed an accumulation of round cells along with some polymorphs, this more particularly in the vicinity of the bile ducts - a peri-cholangitis. In the lumen of some of the bile ducts, polymorphs and inspissated bile were found. Stokes, who first drew attention to the presence of peri-cholangitis, suggested that the jaundice might be attributed to it. Focal necrosis of the liver cells was occasionally observed with a cellular reaction round the periphery. Fatty change was not usually very marked.

Prominence is given by Monti to two changes viz,. fatty/

fatty degeneration and focal necrosis; these tissue alterations, however, were only occasionally noted in this investigation and were of small extent, consequently they were not considered characteristic features of the disease. The same author emphasized the occurrence of mitosis, especially in the bile duct epithelium, this change also was not very noticeable in the liver sections examined by me. Bile pigment was not as a rule present in visible form in the liver cells or sinuses.

The Kidney constantly showed evidence of acute parenchymatous nephritis with the usual accompanying features, and with hyaline, epithelial, and blood casts in the secreting and collecting tubules. The degree of the parenchymal change varied, as in the liver, from moderately acute cloudy swelling to advanced granular disintegration, with almost complete disappearance of the nuclei in many secreting tubules. Haemorrhage into the cortical and medullary interstitium, and into the tubules (Fig. 26), was not an infrequent occurrence and extravasations of blood were noted in the glomerular spaces and beneath the kidney capsule.

Occasionally accumulations of leucocytes were seen in the secreting tubules, and some blood casts contained/

Fig. 25. Disruption of liver parenchyma by haemorrhage and oedema, guinea pig.

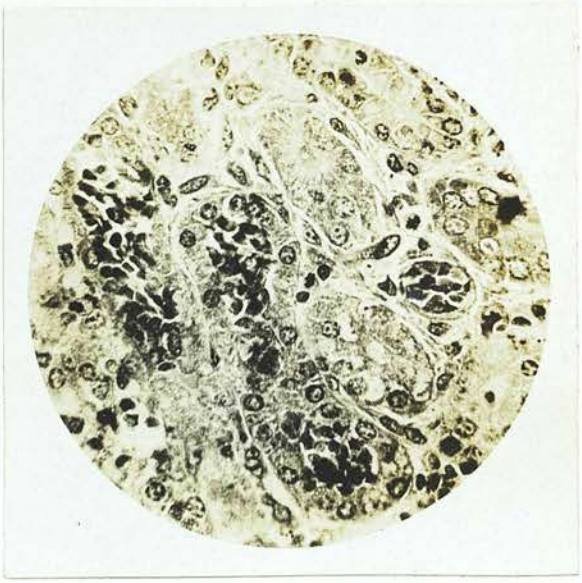


Fig. 26. Haemorrhage into secreting tubules, kidney, guinea pig.

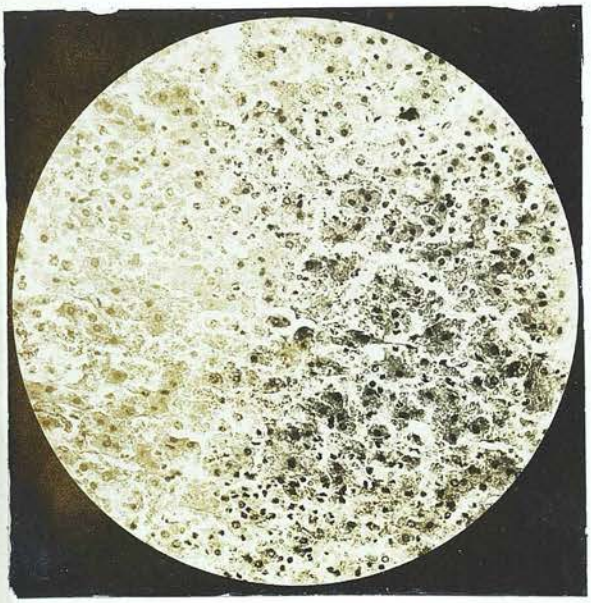


Fig. 27. Suprarenal gland, showing haemorrhage and excess of polymorphs, guinea pig.

Fig. 28. Spleen, phagocytosis of red cells and endothelial cell proliferation, guinea pig.

contained numerous polymorphs and lymphocytes.

The Suprarenal was usually the seat of extensive haemorrhage (Fig. 27), chiefly involving the medulla, but sometimes the whole gland was affected with consequent destruction of cells and normal structure. In the haemorrhagic areas an excess of polymorphs and lymphocytes were found.

The Spleen invariably was markedly enlarged, congested and haemorrhagic: it showed extensive proliferation of endothelial cells, and pronounced phagocytosis of red cells (Fig. 28). In many of the phagocytes pyknotic degeneration of the nuclei was noted, and in other endothelial cells, minus ingested erythrocytes, cell division was observed. Haemosiderin and bile pigment were recognised within some phagocytes but not in great amount.

The Lymph Glands were also the seat of very marked and extensive erythrophagocytosis, (Fig. 32), the picture being almost identical to that of the spleen. Endothelial cell proliferation was also a feature, but haemorrhages were not always present.

The Bone marrow showed slight evidence of red cell phagocytosis.

The Lungs were characterized by discrete haemorrhages (Fig. 29), some situated immediately beneath the pleura, others isolated in the lung substance, or in relation to the bronchioles and vessels. Oedema at times accompanied the haemorrhages. The pulmonary veins and alveolar capillaries were distinctly congested, and there was proliferation of endothelial cells, many of which appeared surchaged with greenish-black bile pigment. Phagocytosis of red cells, was noticeable in the haemorrhagic areas, but in a very minor degree compared to the lymph glands and spleen. In several instances a marked peri-vascular, and peri-bronchial round-cell reaction was observed, and in the alveoli polymorphs and lymphocytes appeared in excess.

The Heart apart from capillary haemorrhages and congestion (Fig. 30), revealed otherwise little of note.

In the Stomach there appeared not infrequently small and large haemorrhages in the submucosa and beneath the peritoneal layer.

The Intestines showed submucous and subperitoneal haemorrhages with extravasations of blood into the lumen/.

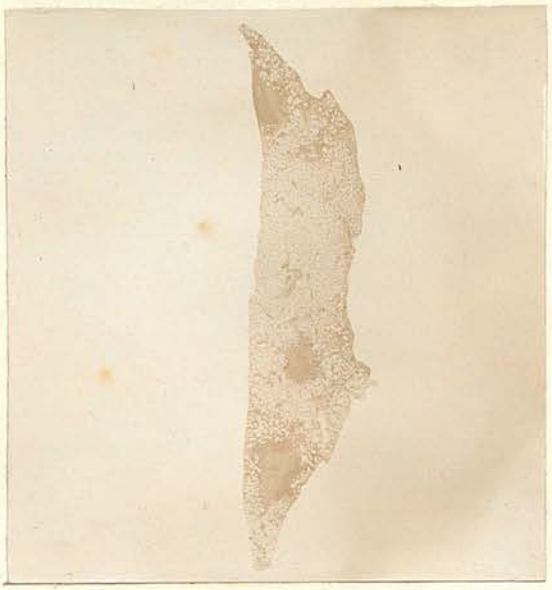


Fig. 29. Guinea pig lung showing small discrete haemorrhagic areas.

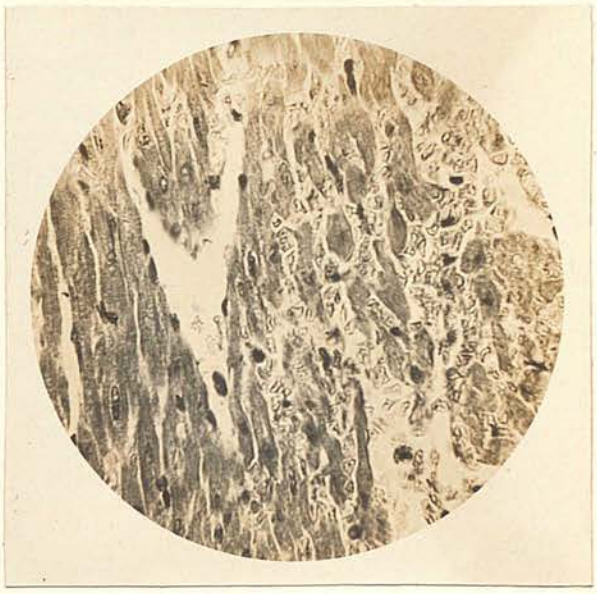


Fig. 30. Capillary haemorrhages heart muscle, guinea pig.

Fig. 31. Haemorrhages in small intestine, guinea pig.

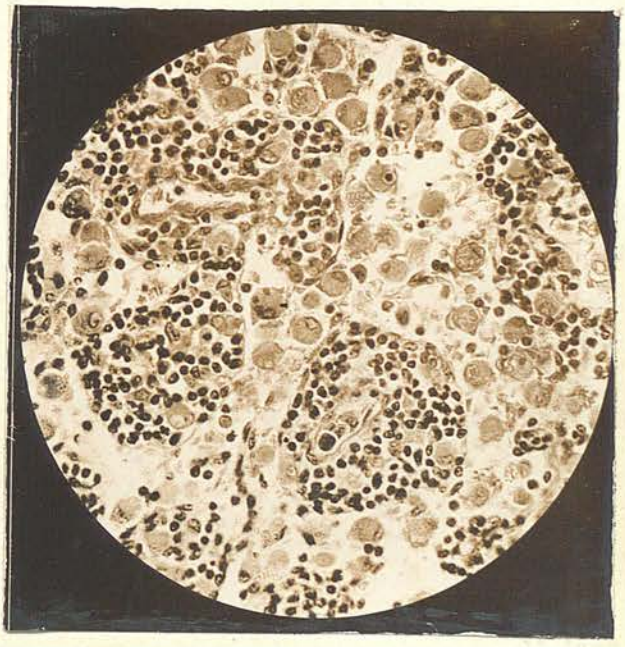


Fig. 32. Phagocytosis of red cells and proliferation of endothelial cells, lymph gland, guinea pig.

lumen. The muscular coat and lymphoid nodules were similarly involved, and proliferation of endothelial cells and erythrophagocytosis were decided features in all the haemorrhagic areas. Distinct phagocytosis of lymphoid cells was also observed, replacement being made by endothelial cells. Plugs of polymorphs and desquamated lining epithelium were present in many of the submucous glands.

The Pancreas was little altered, but small haemorrhages occasionally appeared in this gland.

The Bladder as a rule presented no microscopic changes: in only two instances were haemorrhages found.

The Uterus in several animals showed marked haemorrhages: in one, this organ presented the appearance of a bicornuate blood clot, and in several pregnant animals, microscopic sections of the uterine wall, placenta and embryos revealed extensive haemorrhage into all structures.

The Muscles, particularly of the lower jaw (Fig. 33), and the thigh were the site of numerous capillary haemorrhages.

The Brain in a few cases showed small blood effusions in the pia mater of the medulla.

Certain of the microscopic changes described, merit special note on account of their invariable constancy in the organs and tissues of those guinea pigs which died of typical spirochaetal jaundice. Haemorrhages were found in practically all structures of the animal body. Phagocytosis of red cells was a remarkably pronounced feature, particularly in lymphoid tissues, e.g. the spleen, lymphatic glands, and Peyer's patches. The infection constantly created a conspicuous hyperplasia of endothelial cells in these situations. In fact, Peyer's patches mainly consisted of endothelial cells, and many contained ingested lymphoid elements. Phagocytosis of red cells in the liver and lung tissue is described by Martin and Pettit, but in my experience it was observed only to a very slight extent. In practically all of the animal cases there was no visible bile pigment in the liver and apart from the presence of peri-cholangitis, there was no obvious alteration in the bile ducts to account for the origin of the jaundice. Inada and Kaneko declared that in spite of the intense jaundice in the guinea pig, bile was not precipitated in the liver, and the bile channels possessed normal permeability. This was later confirmed by Brulé and Moreau who expressed their conviction of the integrity of the bile passages.

On/

On the other hand biliary stasis in the liver of the six fatal human cases was evident from the appearance displayed by the microscopic sections of that organ (Fig. 5).

Attention has been drawn to the existence of peri-cholangitis in both human and animal forms of the disease, and to the view expressed by Stokes regarding its possible relation to the occurrence of jaundice. The possibility of a haematogenous origin of the jaundice requires attention also, but in considering the blood-count figures (pp. 42) from this point of view, it will be seen that no appreciable decrease in red cells occurred on the third day after inoculation, when jaundice was usually first noticed in the guinea pigs.

The icterus daily became more intense notwithstanding the fact that the blood counts remained more or less steady until one or at most two days before death.

The figures indicate the occurrence of a terminal and pronounced blood destruction, which no doubt is instrumental in accentuating the jaundice just before death, but evidence is lacking to prove that the icterus is essentially of haemogenous origin. As a first cause of all the tissue changes described, various writers have cited the action of possible toxins, either/

either elaborated by the spirochaete during life, or set free after degeneration of the organism in the tissues. In reviewing the literature, however, there is no evidence of such toxins having been separated from cultures of the organism. This question has not been particularly studied in the present investigation.

Distribution of the Spirochaete in the infected Guinea Pig.

The morphological characters of the organism, as observed in the tissues, are dealt with in a later section (p. 74)

Unlike human infection, the spirochaete was readily found in the blood in guinea pig infection. According to the Japanese observers it appeared in the blood on the 5th or 6th day after inoculation, whereas, Martin and Pettit recorded its presence in the circulation as early as 48 hours after injection. In my own observations, the spirochaete was found in the peripheral circulation, at the earliest, 72 hours after inoculation and even then it was detected with difficulty; its appearance was concomitant with the maximum height of the fever (vide temp. chart, G.P 7, p. 37). In the tissues the number of spirochaetes varied/

Fig. 33. Capillary haemorrhages in muscle of jaw, guinea pig.

Fig. 34. Spirochaetes surrounding individual liver cells, guinea pig. Modified Levaditi method.

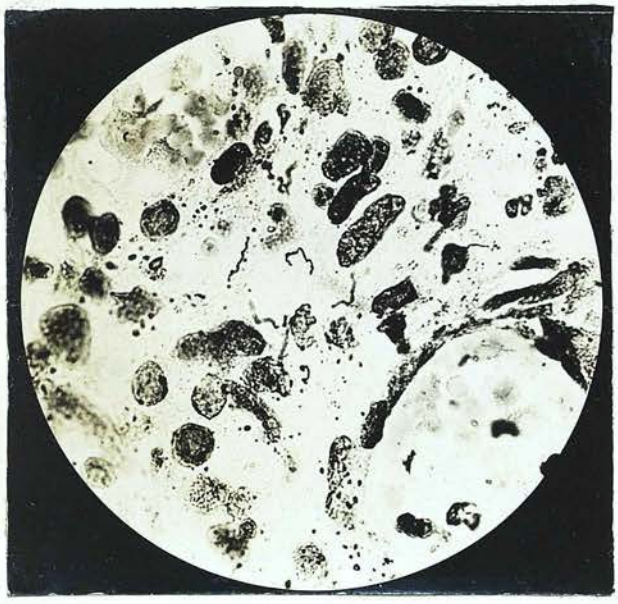


Fig. 35. Spirochaetes in interstitial tissue of kidney, guinea pig. Modified Levaditi method.

Fig. 36. Spirochaetes in capillaries of jaw muscle guinea pig. Modified Levaditi method.

varied considerably in accordance with the organ. The liver usually harboured them in abundance, where they were mostly extracellular and arranged like a garland round individual liver cells (Fig. 34). Intracellular forms were not so rare as certain writers indicate and in some cases it seemed as if spirochaetes might be situated in the bile canaliculi of the liver cells. It is interesting to note that, apropos of the predilection of this spirochaete for the liver tissue, Uhlenhuth and Fromme stated that the liver of a guinea pig 7 hours after inoculation proved "virulent".

The suprarenal as a rule contained many spirochaetes but in no instance were they so numerous as in the liver. In the kidney (Fig. 35), the number varied, but usually they were numerous, especially in the interstitial tissue of the cortex. In the other organs and tissues the spirochaetes were always found to be relatively few, although their presence was observed in almost all parts of the animal body at one time or another, with the exception of the brain.

Costa and Troissier described the presence of spirochaetes also in the central nervous system of the/

the guinea pig, but no satisfactory evidence of this has been personally obtained. The same observers have recorded the penetration of the spirochaete through the animal placenta and the transmission of the disease by inoculation of amniotic fluid; the exclusion of infected blood in such circumstances must be difficult. Numerous microscopic sections of embryonic guinea pigs have been personally examined but no spirochaetes were found, although, as previously mentioned, extensive haemorrhage into the embryonic and placental tissues was a feature. Spirochaetes were frequently found in the urine and occasionally in the peritoneal fluid, but none were ever detected in the bile of the gall bladder.

3. MORPHOLOGY.

The study of spirochaeta icterohaemorrhagiae in the living state, as found in the infected animal body, or in culture, is almost impossible under the ordinary microscope; particularly suitable for this purpose, however, is the dark-ground microscope. The organism may also be studied in film preparations treated with certain stains, or silver nitrate, and in sections of various tissues.

Appearance of the organism by dark-ground illumination.

This procedure was found to surpass all other methods of examining the exact structure of the spirochaete, and overcame the defects of fixatives and stains. With the aid of special slides and coverglasses wet films were prepared from tissue emulsions in saline, from blood, urine, and cultures of the organism, and directly examined under the dark-ground microscope.

The spirochaete assumed the appearance of a bright filament, and exceedingly fine spirals throughout its entire length gave it a distinctly serrated outline. The ends were pointed and frequently curved, and/

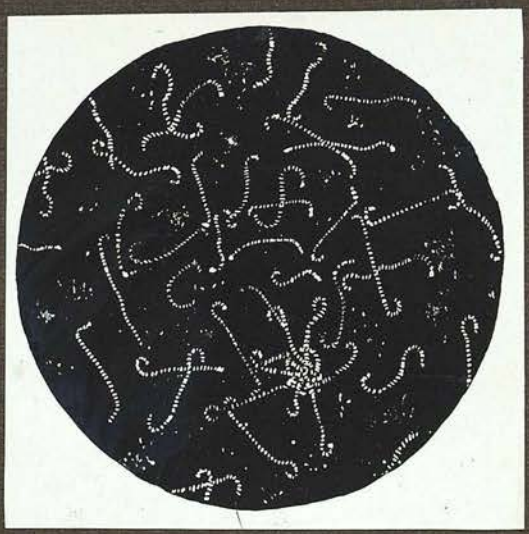


Fig.36A. Appearance of the organism under the dark - ground microscope. X 1000 approx..

Fig.36B. Spirochaeta or leptospira icterohaem - orrhagiae showing the fine spirals as seen by Fontana's method. X 2000 diam..

and forms with C and S incurvations, described by Inada and Ido, were not uncommon (Fig. 36[A]). Unusually long types with several undulations were observed, especially in laboratory cultures. The measurements though not particularly estimated, appeared to approximate the figures reported by Noguchi, and Martin and Pettit; the former compared the Japanese, Belgian and American strains which agreed in dimensions, and the latter reported almost similar figures for the French strain. Noguchi's measurements are as follows: 7 to 9 to 14μ long, exceptionally, 30 - 40μ; diameter, 0.25 - 0.3μ, cylindrical; spiral amplitude, 0.45 - 0.5μ, regular, rigid; 20 spirals in 9μ size, (Martin and Pettit). The following further particulars are also quoted from Noguchi: axial filament not recognised; no chambered structure, membrane, or crista; terminal finely spiral filament not recognised; no flagella: highly motile end portion, well developed in the last 6 to 8 spirals: division transverse. The features of the living spirochaete that arrested attention at a glance, were its singularly unique form of activity in a free state, the striking flexibility of the organism, and its apparent granularity, caused by light refraction from the fine spirals throughout its length. This granular appearance was wrongly interpreted by German observers (Hübener and Reiter), who/

who proposed the name "spirochaeta nodosa" for the organism. Its motility was characterized by extremely rapid rotation on its long axis within the microscopic field, and during this process both ends were usually incurved. In active translation one end straightened, while the other continued revolving in the curved form, and rapid progression was made in the direction of the straight end. When its activities were impeded by tissue elements or semi-solid culture medium in wet films, it affected serpentine movements and the fine spirals in this state appeared more open and stretched. The organism clearly differentiated itself from the so-called "pseudo-spirochaetes", i.e. the delicate filaments frequently noted in haemolysed blood, in dissociated fresh tissue in saline, and in urine containing blood where osmotic change had occurred. The motility of spirochaeta icterohaemorrhagiae was distinctive enough to distinguish it at once from these filaments, whatever their origin, and even if inactive its characteristic morphology was never exactly simulated. This organism on account of its unique structure, differing from all other spirochaetes hitherto described, in possessing fine elementary spirals, was given the representative name of a new genus, Leptospira (leptos, - thin; speira, - coil), created/

created by Noguchi in 1917, the type species of which he named, Leptospira icterohaemorrhagiae (Inada and Ido, 1914). A feature which differentiated it from spironemata and treponemata lay in its complete resistance to the action of 10% saponin (Noguchi). Objections to the acceptance of this new generic title, however, have been made by French writers. In brief, they deprecate the naming of the organism by any but the Japanese discoverers, and disaprove of saying and writing that the causal agent of spirochaetosis icterohaemorrhagica is a leptospira; consequently the French writers adhere to the original nomenclature of the Japanese authors. In this country, however, the organism has been described under both generic names although that created by Noguchi finds more favour.

Technique employed in studying the organism in film and tissue preparations.

The spirochaete may be demonstrated in thin smears of infected animal tissues, or films of body fluids, by drying the preparations in the air, fixing in alcohol, and staining with dyes generally applicable to protozoa, e.g. Giemsa, and Leishman's stain. Dried/

Dried films may also be treated by Fontana's silver nitrate method. Renaux and Wilmaers recommended the use of simple stains, such as, toluidin blue, methylene blue, and fuchsin, on films previously treated with 10% tannin solution. Although the organism was readily demonstrated by all the foregoing methods, it presented diverse aspects according to the technique and, with the exception of Fontana preparations, the fine spirals were either entirely obscured, or only suggested by a distinct granularity in the body of the organism. Films carefully prepared by Fontana's method, or fixed in osmic acid vapour and stained with Giemsa's solution gave very satisfactory pictures of the natural features of the spirochaete.

Among the various methods tried by the writer, the following three were found most suitable for the object in view, namely, that of demonstrating the fine spiral structure.

1. Film preparations were fixed when moist in the vapour of 1% osmic acid solution for 2 minutes. A desiccator was found to serve the purpose admirably. They were then hardened in absolute alcohol 15 - 30 minutes, washed thoroughly in distilled water, and stained overnight in a weak Giemsa solution (2 in 20 dilution), to which/

which a few drops of 2% potassium carbonate solution were added.

2. Fontana's silver impregnation method was found to give excellent results (Fig. 36[B]), if certain precautions were observed in the technique, e.g. filtration of all reagents immediately before use, thorough washing of the film in distilled water after treatment with each reagent, and avoidance of overheating throughout the process.

3. Satisfactory preparations were also obtained by fixation of films in absolute alcohol 15 minutes, thereafter treating with ether to remove fatty material, particularly in liver and kidney smears; this procedure was also applied with advantage to films from other sources. The slides were treated with alcohol again, thoroughly washed in distilled water and stained with weak Giemsa solution as in the first method. After fixation in alcohol the films were not allowed to dry during the process.

The results obtained with Leishman's stain, applied/

applied in the usual manner, were variable, but immediate staining of fresh films prepared from infected guinea pig blood (Fig. 1), yielded very good results, when the stain was allowed to act for 30 minutes in a closed glass cylinder.

The appearance assumed by the spirochaete in films made from the sources mentioned, and carefully fixed and stained as described, invariably yielded results which enabled one to recognise the fine spirals without much difficulty.

Numerous other methods have been devised by various workers (Hollande, Tribondeau and Dubreuil, Legroux, Carageorgiades, etc.), but the one that has met with universal approval is that of Fontana, which merits particular commendation.

The morphological feature of the organism, i.e. the presence of fine elementary spirals, was first described by Noguchi, who at the same time stated that no terminal filament was recognised (vide p. 67). Martin, Pettit and Vaudremer, however, by using Loeffler's, and van Ermengem's flagella stain demonstrated what appeared to be terminal filaments with spherules at the end. They detected them only in films treated by the methods mentioned, and remarked that their presence was not confirmed in the living spirochaete as seen by dark-ground illumination.

Although/

Although these French observers are inclined to the view that the filaments and spherules are part of the organismal structure and not artefacts, still they reserve final judgment until further information has been obtained, and meantime appraise the effect as curious to the reacting agents, particularly those concerned in the formation of silver albuminate, (Van Ermengem's method). The presence of an "end-body" was described by Zuelzer, the relation of which to the organism may be better conveyed by a literal quotation from this writer: "Weil's spirochaete is of the type, sp. plicatilis, and has a spiral wound round a central axial filament, which terminates in an end-body. It forms a special species of the genus, Spirochaeta". Reference to Noguchi's observations on the morphology of this organism (p. 67) indicates the lack of unanimity regarding its finer structure and the genus to which it belongs. The writer has not succeeded in demonstrating terminal filamnets or spherules by the Muir - Pitfield flagella stain.

Technique of tissue preparation for spirochaetes and their appearance in Section.

Various methods of tissue impregnation with silver nitrate have been advocated, and many tried by the writer,/

writer, but the one which gave uniformly excellent results, wholly free from artefacts, was Dobell's modification of Levaditi's method. The procedure is as follows: a piece of tissue 1m.m. thick is fixed in 10% formalin (renewal of the fluid and prolonged fixation enhance the result). It is then placed direct into absolute alcohol and allowed to remain 24 hours. This is followed by passing the tissue through graded alcohols, within a period of 7 - 9 hours, to distilled water in which it is allowed to sink, then removed into a fresh supply. It is then impregnated with a 1% silver nitrate solution for 18-24 hours, thereafter washed thoroughly in repeated changes of distilled water and placed in a 1% solution of Hydroquinone 19 - 24 hours. The specimen is again passed through several changes of distilled water, dehydrated etc., and embedded and cut in paraffin. A further advantage of this method consists in the ready enhancement of the sections by counterstaining.

The appearance of the spirochaete in sections of the various tissues differed considerably from that seen in culture etc., by dark-ground illumination, in film preparation. It presented in most cases a short stumpy form; no fine spirals, terminal filaments, or spherules, (Martin and Pettit), were recognised, but/

but numerous irregular undulations (Fig. 34), similar to those seen in spirochaetes found in the urine (Figs. 48), were much in prominence. Apart from conveying an idea of the numbers and distribution of the organism in the tissues no characters peculiar to this spirochaete were ever defined in sections.

4. Cultivation of the Organism.

The Japanese observers, Inada and Ido, were not only the first to discover the specific spirochaete, but also the first to essay its cultivation in an artificial medium. Partial success was obtained with the culture medium introduced by Noguchi for the growth of treponema pallidum. This they modified however, by substituting guinea pig, for rabbit kidney, in the mixture of ascitic or hydrocele fluid and agar, in which the spirochaetes remained alive 13 - 17 days. At the same time they noted the following important facts: the spirochaete developed better at a temperature below 37°C., it slowly disappeared from culture incubated above 37°C, and the optimum temperature of growth appeared to be between 22° and 25°C..

Ido and Matsuzaki (1916) later introduced two new media, one, in liquid form, consisting of human or ox serum diluted with an equal quantity of distilled water, or ascitic or pleural fluid, the other, a solid medium, composed of agar or gelatin and blood. Satisfactory cultures were apparently obtained. Reiter and Ramme (1916), recommended the use/

use of rabbit's serum in normal saline in the proportion of 1 in 5, and stated that the best cultural results were obtained by inoculating the medium when it was several weeks old. This serum mixture has been varied by other workers some of whom added ox serum, one part, to nine parts of normal saline, Locke's or Ringer's solution. Uhlenhuth (1917), claimed success in cultivating the spirochaete in a simple mixture of rabbit's serum and tap water (1 in 10), and further, merely added one part of infected guinea pig blood to thirty parts of ordinary water. In the absence of contamination, multiplication of the organism is stated to have occurred in most cases. This simple procedure he proposed for the diagnosis of spirochaetal jaundice in man, but I have been unable to trace any reference to its success in the literature consulted. Noguchi (1918), formulated the following media which has been used with success in this investigation:

A. Rabbit's serum. 1.5parts
Ringer's solution 4.5 "
Citrate plasma 1.0 part
Paraffin oil to cover the surface.

B. Rabbit's serum 1.5 parts
Ringer's solution 4.5 "
2% agar 1.0 part
Paraffin oil to cover the surface.

C. Rabbit's serum 1.5 parts)
Ringer's solution 4.5 parts)
2% agar 1.0 part)
(Semisolid portion)

After solidification add:/

add:

Rabbit's serum 1.5 parts)
Ringer's solution 4.5 parts)
Paraffin oil to cover the surface.)
(Fluid portion).

It was found convenient to introduce the rabbit's serum into B and C, when the agar medium in tubes was cooled to about 40°C. Growth is stated to begin much sooner in medium A than in medium B, but after a month more spirochaetes will be found in B. Medium C is claimed to combine the advantages of A. and B.

D. is a medium for acclimated strains:

Horse or ox serum 1 part
Ringer's solution 3 parts.

This proved to be fairly suitable for strains which had become accustomed to the various media (A,B,C), during a period of several months (Noguchi). Another medium devised by the same author for this purpose may also be employed:

Defibrinated rabbit's blood 1cc.
Ringer's solution or normal saline 8cc.
2% agar 1cc.

The blood is added when the agar medium is at a temperature between 40 - 50°C.

Martin and Pettit (1919) preferred the mixture of rabbit serum and normal saline introduced by Reiter and Ramme.

Wenyon (1921), obtained good results with a modified Noguchi serum medium which he employed for the cultivation of leptospira and protozoa. It is prepared as follows:- To 270c.cm. of 0.86 per cent sodium chloride solution are added 30c.cm. of 2 per cent ordinary nutrient agar (pH. 7.6). When mixture has taken place, 10c.cm. are placed in each test tube. After autoclaving at 120°C. the tubes are cooled to 50°C., and into each tube are allowed to drop, from a rabbit's ear, 20 drops of blood. The tubes are not shaken or mixed, and are incubated for twenty-four hours at 37°C. The medium is then ready for use.

The blood is obtained by shaving the ear over the marginal vein, sterilizing the skin with alcoholic iodine solution, and coating above, below, and on the margin of the ear with hot melted paraffin. With a small sharp knife an incision is made in the marginal vein through the thin layer of paraffin wax, and the sterile blood allowed to drop into the culture tubes. After incubation of the blood agar tubes it will be found that the blood has coagulated in a cylindrical column, leaving a clear agar medium around it. There are obvious advantages of this medium over the rabbit's serum media of Noguchi, as it is more easily prepared, and involves no separation of serum from the blood.

The/

The author stated that leptospira icterohaemorrhagiae grew readily in it and that sub-culture required to be made every three weeks. It is interesting to note that Whittingham isolated the leptospira from cases of sandfly fever at Malta in this medium (Wenyon). My personal experience proved it to be favourable for the growth of leptospira icterohaemorrhagiae, but not quite so good for initial cultivation as that of the Noguchi rabbit's serum media.

Akira Shiga)1924), in the course of certain experiments, the results of which are discussed in a later section (p. III), noted that the organism of spirochaetal jaundice grew in colloidal silicic acid without the addition of serum, and another writer Saka described the occurrence of ~~the~~ growth in a simple solution of gum acacia.

Many of the foregoing media were employed in this investigation, but for primary culture of the organism Noguchi's semi-solid medium B, gave much the best and most constant results; although growth occurred to some extent in certain of the media advocated by others, yet it was never so rapid or abundant as in Noguchi's medium.

Another advantage of medium B, much appreciated in this work, was its clarity, which rendered visible the gradual extension of growth from the upper layer of/

Fig.46 Leptospira icterohaemorrhagiae. Appearance of the growth in Noguchi's rabbit serum medium.

Fig. 47. Microscopic appearance of the organism from culture, as seen by dark-ground illumination.

of the medium downwards (Fig.46).

For maintenance of strains acclimated to culture, the medium made up with defibrinated rabbit blood in agar and normal saline (p. 78), was found to answer the purpose admirably. Notable success with either horse, or ox serum in saline was not readily, or constantly achieved.

In the preparation of initial cultures from infected animals the writer by preference used the clear serum medium (B), of Noguchi, and in order to observe growth of the spirochaete without continual resort to dark-ground examination, the medium was kept clear by introducing about 0.1cc. of infected tissue emulsion in saline e.g. kidney of rat, and preferably liver of guinea pig.

It may be mentioned that human serum, or that of the horse, ox, guinea pig, and rat did not prove the equal of rabbit's serum or blood, in maintaining the spirochaete in sub-culture. This is rather remarkable considering that the rabbit is more or less refractory to infection by this organism (p. 33). The sera of numerous other animals tested by Noguchi, were also found to be less favourable than that of the rabbit.

Conditions of Cultivation: concentration of serum, reaction of medium, temperature and oxygen requirements.

Noguchi, by experiment, arrived at the following conclusions/

conclusions regarding the factors requisite for suitable growth of the leptospira.

It was found that luxuriant growth occurred in a medium of Ringer's solution to which more than 10 per cent of normal rabbit's serum was added. There was only a moderate growth with 5 per cent, and none when less than 2 per cent was present.

The reaction of the medium was found to be an important factor in the cultivation of the organism. It grew best in a medium which was slightly alkaline (pH. 7.6), not exceeding that of the serum. If the reaction was neutral, growth was meagre, and the organism short lived. No growth occurred when the medium was either acid or distinctly alkaline.

The organism thrived at any temperature between 37° and 10°C., the optimum zone being 30° - 37°C. Growth proceeded more rapidly at 37°C, than at 30° or at 25°, but the cultures remained viable much longer at the latter temperatures. No growth took place at 42°C..

Noguchi described the organism as an obligatory aerobe. He considered that any hindrance to the access of axygen constituted an unfavourable factor in/

in obtaining a culture , notwithstanding the fact that sealing of the cultures with liquid paraffin was advised.

In later culture work the present writer dispensed with the paraffin seal, the absence of which made no marked difference either in the rate of growth, or in its abundance: moreover cultural manipulation was much better performed without the presence of oil. That the organism is an obligatory aerobe is questioned by certain French observers, because of its favourable growth under sealed conditions partially excluding the access of oxygen.

The cultural conditions, fully investigated by Noguchi, and outlined above, were only confirmed generally in the course of the present work. The procedure recommended by this author hardly ever failed to produce the desired results, and primary cultivation of the organism was more constantly successful in Noguchi medium than in any other. The development of growth in primary cultures incubated at 37°C., was clearly recognised, usually at the end of the first week, by a distinct haziness just below the surface of the medium. When growth was visibly established at this time cultures were then kept at 25°C., in order to maintain their viability for a longer period, which in some instances extended to three months.

The/

The organism was maintained in sub-culture by inoculating ordinary tubes or long-necked small flasks containing Noguchi's medium (B), or defibrinated rabbit blood medium; sub-inoculations were made, as a rule, every month or six weeks, and the cultures were incubated and kept at a temperature of 25°C.

Although the virulence of the human and rat strains in culture was not comparatively tested by animal inoculations, still it is desired to record that 1cc. of a rat strain culture (R. 40), eight months old, produced typical spirochaetal jaundice in guinea pigs with a fatal issue in 6 - 7 days (vide p. 43).

Filterability of the organism.

Diverse results have been recorded regarding the passage of this organism through Berkefeld, and Chamberland filters.

Inada and Ido were the first to test its filterability, and used for the purpose Berkefeld V, N, and W grades. The material tested was infected guinea pig liver emulsified in saline. They reported that 15 out of 28 guinea pigs inoculated with the filtrate furnished a positive result. They were unable to detect the organism in filtrate preparations under the dark-ground microscope. Martin and Pettit used Chamberland L filters, and reported results which, they stated, were

Comparable /

comparable to those of the Japanese observers. On the other hand Cesa-Bianchi, and Handel, Ungermann and Jaenisch failed to confirm the filterability of the organism.

The writer carried out experiments to obtain information on this point. In four instances infected liver emulsion in saline was filtered through a Berkefeld N filter, previously tested, and found to prevent the passage of B.coli communis. Four guinea pigs were inoculated with 4 c.c. of the filtrate, but the results were negative. No spirochaetes were detected in several wet films made in each case from filtrate which had been centrifuged. In two instances rich laboratory cultures of the organism in Noguchi's medium were thinned with saline, and passed through a new form of filter called the Seitz-Werke, which prevented the passage of B.melitensis. Again no spirochaetes were found in the filtrate under the microscope, and animal inoculations proved negative, but in one tube of Noguchi's medium inoculated with about 1c.c. of the spun filtrate, leptospirae were found at the end of three weeks .

As the results in the hands of the different workers have varied, the general opinion regarding the filterability of this organism is at present not unanimous.

Viability of the organism in Soil and Water.

The elimination of the organism in the urine of the rat host, suggested an enquiry into the fate of the leptospira thus liberated into natural environments, such as soil and water.

Several experiments were carried out in the following manner. A guinea pig liver rich in leptospirae was emulsified in saline. About 0.5cc. of the supernatant emulsion was introduced into each of twelve different test-tubes containing pit water, fungal slime from the roof of a coal-mine, wet ground soil from a mine, ordinary tap water, distilled water and normal saline. Each particular sample of water, slime and soil used was previously examined for leptospirae, but none were detected by the dark-ground microscope. The inoculated tubes were kept at a temperature which approximated that of the ground in coal-mines during the winter, viz. 12°C. Without entering into details it may be mentioned that in the ground-soil culture, motile leptospirae (2 - 3 in a microscopic field) were found by dark-ground examination up to the 95th day after inoculation. Guinea pig inoculation at this time with about 1 c.c. of the wet/

wet soil did not produce infection. In two tubes containing different samples of pit water leptospirae were found feebly motile however, up to the 75th day after inoculation. The reaction of the water and soil in these tubes remained slightly alkaline, whereas in the other nine tubes, the slime and water medium became acid and overgrown with other organisms, after the 4th day at 12°C.; no leptospirae were ever recognised in these cultures, after the 4th day of inoculation.

Noguchi described their viability as of short duration, not longer than three days, in polluted waters, sewage and soil.

The present writer however, has proved beyond doubt that pathogenic leptospirae may survive for months in a suitable natural environment, but the question of maintained pathogenicity in such conditions has not been definitely decided.

IV. MODE OF SPREAD OF THE DISEASE.

1. Rat Investigation.

The question of animal reservoirs of the organism was solved for the first time by Miyajima in 1915. This observer called attention to the finding of spirochaetes resembling spirochaeta icterohaemorrhagiae in the kidneys of the field mouse, Microtus Montebelloi. According to Ido, Hoki, Ito and Wani (1917), the discoverer, in 1916, reproduced spirochaetal jaundice in the guinea pig by inoculation of infected kidney of the field mouse. As a result of this discovery the foregoing Japanese co-workers examined a number of wild rats, and reported the presence of a similar spirochaete, which also reproduced the disease on inoculation into guinea pigs. Virulent spirochaetes were found in the kidney or urine in 40.2 per cent out of 149 rats, type, mus decumanus, and in 0.8 per cent out of 24 rats, type, mus alexandrinus. Their observations were carried out by means of the dark-ground microscope and guinea pig inoculations. They did not at any time find spirochaetes in the blood, liver, or intestinal contents of the rats harbouring the organism in the kidney or urine, and all animal experiments/

experiments with the blood, liver, and intestinal contents of infected rats were negative..

Since this important discovery was announced the fact has been established that the wild rat (Rattus norvegicus particularly) in most countries acts as an animal reservoir of spirochaeta, or leptospira icterohaemorrhagiae. Stokes, Ryle and Tytler (1917), and Renaux (1917) proved this in Belgium, Martin and Pettit (1917) in Northern France, and Da Silva (1922), and Sigalas and Pirot (1922) in Southern France. In different parts of Italy rat infection with leptospira was reported by Monti (1917), and Grasso (1918); in the United States by Jobbling and Eggstein (1917), and Noguchi (1917), and in South America by Arago (1917). In North Africa, Nicolle and Blanc (1918) found infected rats in Tunis, and Lhéritier (1918) in Algiers, and in Spain similar observations were recorded by Dalmau (1918). Coles (1918) was the first to report the existence of infected rats in this country at Bournemouth. Stevenson (1922) carried out an examination of 100 rats from different parts of England, and reported 30 per cent infected with leptospira icterohaemorrhagiae. Anigstein (1923) described the presence of the organism in rats at Warsaw, Bonne (1924), and Kuenen (1924) in those of Amsterdam, and the writer (1924) recorded the existence of infected rats in Scotland. Several other observers have reported similar findings in the rats of other

countries, but the foregoing references are sufficient to indicate the wide spread distribution of this particular host.

Subsequent to the discovery, in January 1924, of leptospiral infection in two out of three wild rats (p.3) sent to me from a local colliery, through arrangements kindly made by Professor G. Lovell Gulland, an extensive examination of the species in Scotland was undertaken. This was accomplished by making provision with the Scottish Board of Health, the Public Health Department, Edinburgh, and the Scottish Mines Department, to all of which bodies I am indebted for the material which has enabled me to carry out the investigation.

The total number of wild rats examined was 166, two of these were of the black variety, Rattus rattus, and 164 of the brown type, Rattus norvegicus.

In most instances they were received alive, and after killing by means of chloroform, they were immediately examined. With the exception of two, which showed a greyish mucoid condition of the lungs associated with the presence of a large bacillus, all the rats appeared well nourished, and no signs of disease were noted in the kidney or any other organ.

At/

At the beginning of the rat investigation preparations of all the organs, the blood and the urine were subjected to examination for leptospira by the dark-ground procedure. The organism however, when present was found only in the kidney or urine, which confirms the observations of Ido, Hoki, Ito and Wani, (p.88). Consequently the search for leptospira principally confined itself to the examination of the kidney and urine by the dark-ground method already described (p. 66). The dark-ground observations were always controlled by inoculation of guinea pigs, when these were available, with 2cc. of kidney emulsion in saline. The tabulated protocols present the detailed results of dark-ground examination of rat kidney and urine for the leptospira, and of guinea pig inoculation with kidney emulsion in saline.

The following summarizes the results:

Total Number of Rats.	Positive.	Per cent infected.
166	61	36.7

Of the total, 102 were from the Edinburgh area, 30 were psoitive, i.e. 29.4% infected with virulent leptospira. The counties in Scotland in which infected rats were found include, East and West Lothian, Midlothian, Fife, Ayrshire, Perthshire, and Aberdeenshire (Smith).

In/

Figs. 37, 38. Clumps of leptospira icterohaemorrhagiae radially disposed in convoluted tubules of kidney, wild rat. Prepared by Dobell's modification of Levaditi's method.

Figs. 39, 40 Clumps of leptospira icterohaemorrhagiae in urine of wild rat. Giemsa's stain.

In addition to the examination of rats, two field mice, two house mice, a sick cat associated with persons who had spirochaetal jaundice, and a silver fox with slight icterus were examined for the organism. Among these six animals, one field mouse proved to be infected with leptospira icterohaemorrhagiae.

The results of the examination and animal experiments are also tabulated (p. 100).

Incidental to the search for the leptospira in these animals it is of interest to record that the spirochaete of rat-bite fever (sp. morsus muris) was demonstrated in the blood of one rat, (R. 75), and one field mouse, and that by inoculation of blood from these animals the infection was communicated to guinea pigs.

DATE	REF. NO. RAT	DISTRICT IN WHICH RAT CAUGHT.	LEPTOSPIRAE IN KIDNEY OR URINE.	DATE OF GUINEA PIG INOCULA-:TIONS WITH KIDNEY EMULSION.	DATE GUINEA PIG DIED.	RESULT OF GUINEA PIG INOCULATION.		
						Jaundice	Haems.	Leptospirae
23:1:24	R.S.	E.Lothian	+	23:1:24	30:1:24	+	+	+
"	R.P1.	" "	+	"	5:2:24	+	+	+
"	R.P2.	" "	-	"	-	Remained alive and well.		
16:2:24	1.	" "	+	17:2:24	19:3:24	-	-	-
3:3:24	2.	" "	-	3:3:24	-	Remained alive and well.		
6:3:24	3.	Midlothian	+	6:3:24	17:3:24	+	+	+
"	4.	Midlothian	-	"	-	Remained alive and well.		
8:3:24	5.	E.Lothian	-	9:3:24	19:3:24	+	+	+
"	6.	" "	-	"	16:3:24	-	-	-
"	7.	" "	-	"	19:3:24	+	+	+
"	8.	W.Lothian	-	"	-	Remained alive and well.		
"	9.	" "	-	"	21:5:24	-	-	-
10:3:24	10.	" "	+	10:3:24	22:3:24	+	+	+
11:3:24	11.	E. "	-	11:3:24	27:3:24	-	-	-
"	12.	" "	-	"	31:3:24	-	-	-
14:3:24	13.	" "	+	14:3:24	17:3:24	-	-	-
"	14.	" "	+	"	22:3:24	+	+	+
"	15.	" "	-	15:3:24	-	Remained alive and well.		
"	16.	" "	+	"	25:3:24	+	+	+
17:3:24	17.	" "	-	19:3:24	28:3:24	-	-	-
19:3:24	18.	" "	+	20:3:24	29:3:24	+	+	+
"	19.	" "	-	"	-	Remained alive and well.		

Died 3rd day: unknown cause

DATE	REF. No. RAT	DISTRICT IN WHICH RAT CAUGHT.	LEPTOSPIRAE IN KIDNEY OR URINE.	DATE OF GUINEA PIG INOCULA-:TIONS WITH KIDNEY EMULSION.	DATE GUINEA PIG DIED.	RESULT OF GUINEA PIG INOCULATION.		
						Jaundice	Haems.	Lepto-:spirae.
24:3:24	20.	E.Lothian	-	25:3:24	-	Remained alive and well.		
2:5:24	21.	W.Lothian	+	2:5:24	27:8:24	-	-	-
3:5:24	22.	" "	-	3:5:24	-	Remained alive and well.		
22:5:24	23.	" "	+	22:5:24	2:6:24	+	+	+
				" " "	a 2nd G.P. remained alive and well.			
27:8:24	24.	Edinburgh	-	28:8:24	-	Remained alive and well.		
28:8:24	25.	"	-	"	-	Remained alive and well.		
1:9:24	26.	E.Lothian	+					
"	27.	" "	+			No animals inoculated.		
"	28.	" "	-					
"	29.	" "	+					
24:10:24	30.	Edinburgh	-	24:10:24	5:11:24	+	+	+
28:10:24	31.	"	-	29:10:24	3:1:25	-	-	-
29:10:24	32.	"	-	"	-	Remained alive and well.		
"	33.	"	+	"	11:11:24	+	+	+
"	34.	"	-	30:10:24	9:11:24	+	+	+
"	35.	"	+	"	19:2:25	-	+	-
"	36.	"	-	"	8:11:24	-	+	-
30:10:24	37.	"	-	8:11:24	12:11:24	-	-	-
31:10:24	38.	E.Lothian	+					
"	39.	" "	-			No Guinea Pigs inoculated.		
"	40.	" "	+					

DATE	REF. No. RAT	DISTRICT IN WHICH RAT CAUGHT.	LEPTOSPIRAE IN KIDNEY OR URINE.	DATE OF GUINEA PIG INOCULATIONS WITH KIDNEY EMULSION.	DATE GUINEA PIG DIED.	RESULT OF GUINEA PIG INOCULATION.		
						Jaundice	Haems.	Leptospirae.
1:11:24	41.	Edinburgh	-	1:11:24	-	Remained alive and well.		
"	42.	"	-	"	13:11:24	+	+	+
"	43.	"	-	"	-	Remained alive and well.		
4:11:24	44.	"	-	4:11:24	-	Remained alive and well.		
"	45.	"	-					
11:11:24	46.	"	-	11:11:24	-	Remained alive and well.		
"	47.	"	-	"	-	Remained alive and well.		
"	48.	"	-	"	20:11:24	-	-	-
"	49.	"	-	"	1:12:24	-	-	-
"	50.	"	-	12:11:24	-	Remained alive and well.		
"	51.	"	-	"	-	Remained alive and well.		
"	52.	"	-	12:11:24	-	Remained alive and well.		
"	53.	"	-					
"	54.	"	-					
"	55.	"	-					
12:11:24	56.	"	-	12:11:24	12:12:24	-	-	-
15:11:24	57.	"	-	15:11:24	-	Remained alive and well.		
19:11:24	58.	"	-	19:11:24	4:12:24	-	-	-
20:11:24	59.	"	-	20:11:24	16:12:24	-	-	-
22:11:24	60.	"	-	24:11:24	-	Remained alive and well.		
24:11:24	61.	"	-	25:11:24	-	Remained alive and well.		
25:11:24	62.	"	-	26:11:24	10:12:24	-	-	-

DATE	REF. No. RAT	DISTRICT IN WHICH RAT CAUGHT.	LEPTOSPIRAE IN KIDNEY OR URINE.	DATE OF GUINEA PIG INOCULA-:TIONS WITH KIDNEY EMULSION.	DATE GUINEA PIG DIED.	RESULT OF GUINEA PIG INOCULATION.		
						Jaundice	Haems.	Lepto-:spirae.
26:11:24	63.	Edinburgh	-	27:11:24	Escaped from cage 15:12:24.			
"	64.	"	-	"				
1:12:24	65.	"	+	1:12:24	13:12:24	+	+	+
2:12:24	66.	"	-	2:12:24	11:12:24	+	+	+
"	67.	"	+	-	-	No animals inoculated.		
3:12:24	68.	"	+	3:12:24	17:12:24	+	+	-
"	69.	"	-	"	31:1:25	-	-	-
"	70.	"	-	"	23:2:25	-	-	-
"	71.	"	-	"	14:2:25	-	-	-
"	72.	"	+	"	23:2:25	-	+	-
"	73.	"	+	"	12:12:24	+	+	+
4:12:24	74.	"	-	4:12:24	23:2:25	-	-	-
"	75.	"	-	"	13:1:25	-	-	Sp. morsus muris found in blood.
"	76.	"	+	"	12:12:24	+	+	+
"	77.	"	+	"	14:12:24	+	+	+
"	78.	"	+	"	24:3:25	-	+	-
"	79.	"	-	"	16:2:25	-	+	+ Died 74th day.
"	80.	"	+	"	13:12:24	+	+	+
"	81.	"	+	"	"	+	+	+
"	82.	"	+	"	14:12:24	+	+	+
"	83.	"	+	5:12:24	7:1:25	-	-	-
"	84.	"	+	"	17:1:25	-	+	+ Died 43rd day.

DATE	REF. No. RAT	DISTRICT IN WHICH RAT CAUGHT.	LEPTOSPIRAE IN KIDNEY OR URINE.	DATE OF GUINEA PIG INOCULA-:TIONS WITH KIDNEY EMULSION.	DATE GUINEA PIG DIED.	RESULT OF GUINEA PIG INOCULATION.		
						Jaundice	Haems.	Lepto-:spirae.
4:12:24	85.	Edinburgh	-	5:12:24	19:2:25	-	-	-
5:12:24	86.	"	-	"	23:2:25	-	-	-
11:12:24	87.	"	+	11:12:24	19:12:24	-	+	-
13:12:24	88.	"	-	13:12:24	20:2:25	-	-	-
"	89.	"	+	"	6:1:25	-	-	+
19:12:24	90.	"	-	19:12:24	20:12:24	-	-	-
30:12:24	91.	Fife	+	30:12:24	20: 2:24	-	+ Died 55th day.	-
"	92.	"	-	"	7:2:25	-	-	-
26:1:25	93.	Edinburgh	+	26:1:25	3:2:25	+	+	+
29:1:25	94.	"	-	29:1:25	9:2:25	+	+	+
3:2:25	95.	Fife	-	3:2:25	7:2:25	-	-	-
"	96.	"	+	"	10:2:25	-	+	+
18:2:25	97.	Ayrshire	-	18:2:25	23:2:25	-	-	-
"	98.	"	-	"	3:3:25	+	+	+
20:2:25	99.	"	-	No Guinea Pigs available.				
25:2:25	100.	"	+	25:2:25	1:4:25	-	+ Died 35th day.	+
"	101.	"	-	1 G.P. inoculated 25:2:25	-	Remained alive and well.		
"	102.	"	-					
3:3:25	103.	Edinburgh	-	3:3:25	3:4:25	-	-	-
13:3:25	104.	"	-	No Guinea Pigs available.				
"	105.	"	-					
"	106.	"	-					

DATE	REF. No. RAT	DISTRICT IN WHICH RAT CAUGHT.	LEPTOSPIRAE IN KIDNEY OR URINE.	DATE OF GUINEA PIG INOCULA-:TIONS WITH KIDNEY EMULSION.	DATE GUINEA PIG DIED.	RESULT OF GUINEA PIG INOCULATION.		
						Jaundice	Haems.	Lepto-:spirae.
13:3:25	107.	Edinburgh	-	No Guinea Pigs available.				
"	108.	"	-					
"	109.	"	+					
"	110.	"	+					
"	111.	"	+					
"	112.	"	-					
"	113.	"	-					
"	114.	"	-					
16:3:25	115.	"	-					
"	116.	"	-					
19:3:25	117.	"	-	19:3:25	-	Remained alive and well.		
20:3:25	118.	"	-	20:3:25	3:5:25	-	-	-
"	119.	"	-	"	19:5:25	-	-	-
26:3:25	120.	Banffshire	-	26:3:25	-	Remained alive and well.		
"	121.	"	-	"	-	Remained alive and well.		
2:4:25	122.	Edinburgh	-	2:4:25	7:5:25	-	-	-
"	123.	Fife	+	"	11:4:25	-	-	-
3:4:25	124.	Edinburgh	-	3:4:25	-	Remained alive and well.		
"	125.	"	-					
"	126.	"	-	3:4:25	-	Remained alive and well.		
"	127.	"	-					
"	128.	"	-	3:4:25	25:4:25	-	-	-
"	129.	"	-					

DATE	REF. No. RAT	DISTRICT IN WHICH RAT CAUGHT.	LEPTOSPIRAE IN KIDNEY OR URINE.	DATE OF GUINEA PIG INOCULA-:TIONS WITH KIDNEY EMULSION.	DATE GUINEA PIG DIED.	RESULT OF GUINEA PIG INOCULATION.		
						Jaundice	Haems.	Lepto-:spirae.
3:4:25	130.	Edinburgh	-	3:4:25	-	Remained alive and well.		
"	131.	"	-					
4:4:25	132.	Fife	+	4:4:25	-	Remained alive and well.		
"	133.	"	-	"	-	Remained alive and well.		
"	134.	"	+	"	11:4:25	+	+	+
"	135.	"	-	"	-	Remained alive and well.		
7:4:25	136.	Edinburgh	-	No Guinea Pigs available.				
9:4:25	137.	"	+					
"	138.	"	-	9:4:25	16:4:25	-	-	-
"	139.	"	+	"	19:4:25	+	+	+
"	140.	"	-	11:4:25	-	Remained alive and well.		
"	141.	"	-	"	2:6:25	-	-	-
21:4:25	142.	Perthshire	-	21:4:25	1:5:25	+	+	+
"	143.	"	+	"	3:5:25	+	+	+
"	144.	"	+	"	29:4:25	+	+	+
"	145.	Edinburgh	-	"	-	Remained alive and well.		
22:4:25	146.	Perthshire	-	22:4:25	11:5:25	+	+	+
25:4:25	147.	From Ship, Leith.	-	No Guinea Pigs inoculated.				
"	148.	"	-					
5:5:25	149.	Berwick-:shire.	-	5:5:25	-	Remained alive and well.		
16:5:25	150.	"	-	16:5:25	1:6:25	-	-	-
25:5:25	151.	Forfar-:shire.	-	25:5:25	23:8:25	-	-	-

DATE	REF. No. RAT	DISTRICT IN WHICH RAT CAUGHT.	LEPTOSPIRAE IN KIDNEY OR URINE.	DATE OF GUINEA PIG INOCULATIONS WITH KIDNEY EMULSION.	DATE GUINEA PIG DIED.	RESULT OF GUINEA PIG INOCULATION.		
						Jaundice	Haems.	Leptospirae.
27:5:25	152.	Edinburgh	-	27:5:25	-	Remained alive and well.		
29:5:25	153.	E.Lothian	-	29:5:25	10:6:25	+	+	+
"	154.	"	-	"	"	+	+	+
28:8:25	155.	Edinburgh	-	29:8:25	-	Remained alive and well.		
"	156.	"	-	"	-	"	" "	"
"	157.	"	-	"	-	"	" "	"
"	158.	"	-	"	-	"	" "	"
"	159.	"	-	"	-	"	" "	"
"	160.	"	-	"	-	"	" "	"
22:1:25	161.	Banff	-					
"	162.	"	-	22:1:25	-	"	" "	"
"	163.	"	-					
	OTHER ANIMALS EXAMINED FOR PRESENCE OF LEPTOSPIRAE.							
20:2:24	1.	Field Mouse, E.Lothian	-	21:2:24	-	Remained alive and well.		
"	2.	"	-	"	29:3:24	+	+	+
28:8:24	3.	House Mouse Edinburgh	-	28:8:24	-	Remained alive and well.		
"	4.	"	-	"	-	"	" "	"
16:8:24	5.	Cat from infected house, Edinburgh	-	4 G.P. inocd. 16:8:24	-	All remained alive and well.		
1:8:25	6.	Silver Fox, E.Lothian	-	3 G.P. inocd. 1:8:25.	-	" "	"	" "

2. Water Investigation.

The examination of water from coal-mines in East Lothian, where spirochaetal jaundice had occurred, was suggested on becoming acquainted with certain literature. Wolbach and Binger (1914), recorded the presence of a spirochaete, which they named spirochaeta biflexa, in a fresh water pond. The description and illustrations of the organism, in the light of recent work on the subject, identify it at once with the genus, Leptospira, created by Noguchi. In 1922 Zuelzer discovered in tap-water a spirochaete, morphologically similar to that of Weil's disease or spirochaetal jaundice. Within the last year or two other workers have reported similar findings, which are discussed later, and more appropriately, relative to a discovery made by the writer. The original observation is of considerable importance in relation to the occurrence of spirochaetal jaundice among coal-miners. The following account indicates the circumstances associated with the discovery, with a discussion on the significance of the observation.

A personal visit to one of the infected coal mines was arranged by the county medical officer of health for East Lothian, Dr G. Y. Richardson, who, by the kindness of the pit owner, received permission for an inspection in February, 1924. It so happened that the mine visited was inundated with water in certain parts, and was known as a "wet" pit. A large number of water samples was collected, by means of sterile glassware, from different sections of the mine. These samples included/

included specimens from new cuttings where the miners were working practically under water, from recent workings which were very wet, and from old sections which may be termed damp in comparison with the other parts. It was in one of the old sections that the following observations were made which resulted in a finding of much significance.

Compared with other branches of the mine there was little ground water; no water was seen to drop from the roof, but the whole tunnel was very damp. Small stagnant, muddy pools were present from which samples were collected. Roof water was looked for in vain, but on holding a lamp close to the tunnel roof I noticed a slimy viscid substance, which formed a small thin layer only in certain areas. At parts it was seen to form hanging drops, which as they increased in size would no doubt fall into water or on to the ground. Other portions of the roof in the same section were merely damp with no slime, while in certain areas red sandstone drippings were present. The viscid substance was certainly not plentiful, and even the small amount collected was found only after careful search.

Wet films of the slime were first examined by dark-ground illumination, which revealed remarkable and/

and varied forms of motile organisms, in addition to primitive forms of the vegetable kingdom. Different types of spirochaetes were distinguished by their morphology and motility. Only after prolonged examination were definite leptospiral organisms found, but they were few in number. The difficulty in recognising this delicate organism in such a viscid substance may be emphasized.

To control the observation if possible, two guinea pigs were inoculated intraperitoneally with 2c.cm. of the glutinous material, thinned by the addition of a little saline to permit of its passage through a syringe needle. The result of these experiments was negative in both cases; no spirochaetosis developed during one month's observation.

A fresh sample of the roof slime from the same section of the mine was obtained at a later date. Dark-ground examination again showed the undoubted presence of typical leptospirae (Fig.41) Two guinea pigs were again inoculated, but on this occasion with 4c.cm. of the substance, as the failure to infect the first two was attributed to the scarcity of the leptospirae in the smaller amount injected. Both animals died, one on the eleventh, the other on the thirteenth day after/

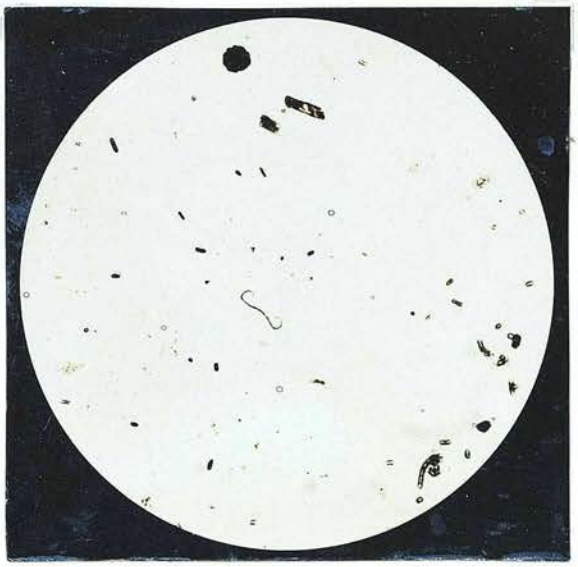


Fig. 41. Water leptospira, × 1000 diam. (slime coal-mine) indistinguishable from human and rat types. Stained by Fontana's method.

Fig. 42. A larger type of water ?leptospira, × 1000 diam. (slime coal-mine) Spirals small and regular but coarser than Fig.41, and ends not pointed. Stained by Fontana's method.

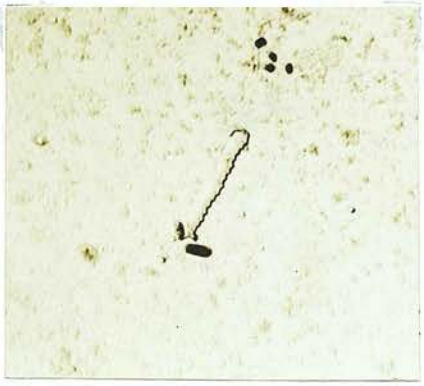


Fig. 43. Spirochaeta stenostrepta, × 1000 diam. (slime coal-mine) free living organism common in fresh and marine water. Stained by Fontana's method.

Fig. 44. Nematode, fungal filaments etc, in slime from roof of coal-mine.

after inoculation. They showed equally pronounced evidence of spirochaetal jaundice. Abundant laptospiral organisms were present in all the organs examined, and an appreciable number was also found in the blood and urine. Notable features were the intensity of the jaundice, the prominence of the widespread subcutaneous, muscular, and internal haemorrhages, the enlargement of the spleen, and the haemorrhages in the stomach in both animals. In fact, the lesions appeared more pronounced than in many animals infected with the human or rat strains.

It would appear, as far as can be ascertained from the relevant literature consulted, that this is the first occasion on which the direct inoculation of a water leptospira has proved pathogenic to guinea pigs. This infected roof slime was found growing on slaty stone and wooden roof-bars in absolute darkness. It was greyish-brown in colour, and translucent when free from carbon particles. It formed a mass of considerable coherence and was not very readily detached from the stone roof. The consistency approached that of a thick gum solution, and resembled the semi-solid culture medium of Noguchi used in growing leptospira. The temperature of the slime in the mine/

mine was 11.7°C. in February. It was slightly alkaline when tested with litmus paper, and had a reaction of pH = 7.5 approximately.

As mentioned above, the leptospira was recognised with difficulty in this substance teeming with other active life. A few of them were much longer than those found in man, the rat, or in cultures. Other types of spirochaetes were much more numerous, particularly one species. The characteristic motility of the leptospira in contrast to that of the other spirochaetes present was striking, and formed a feature which distinguished them from forms closely resembling the genus. Most of the other spirochaetes on escaping from the slime were actively translatory and moved rapidly in a horizontal plane; whereas the true leptospira remained in the field actively rotating for a second or two, then finally burrowed vertically into the viscid medium out of sight. The leptospira was found to exist in this semi-solid medium symbiotically with algae, fungi, cocci, bacilli, spirochaetes, flagellates, nematodes, and their ova. This slimy substance was formed by organisms with a glutinous envelope which yielded a jelly-like or zoogloea mass.

The continued existence of the leptospirae and the other motile organisms was noted at different temperatures./

temperatures. Some tubes of slime were kept at room temperature, and others in the ice-chest for over five months. Dark-ground examination at the end of that time showed all the motile organisms including leptospirae, quite recognizable and unimpaired in their activities. Animal inoculations were recently done to determine if the slime after keeping had maintained its infectivity. No definite results, so far, have been obtained.

It was previously noted that the slime was observed only in a very damp and old section of the mine; its situation on the roof appeared inaccessible to rats. I was informed by the pit manager that at the time of the outbreak of jaundice in May 1923, practically all the miners affected were working in a particular section. This section was stated to have been the wettest part of the mine. Of much significance becomes the fact that this particular section happened to be that branch of the mine in which the infected roof slime was found. Eight miners developed symptoms of spirochaetal jaundice when engaged in the mine in question. Five of these were said to have fallen ill within a period of two weeks while working in this very wet cutting. Rats were never considered numerous in the pit, and an expert rat-catcher failed to trap/

trap any underground for examination, but caught one on the pit surface. This rat was found to be infected with pathogenic laptospira.

It is highly probable that such slime with leptospirae aggregated in it may infect by various routes. At the time of cutting into a new wet section existing slime in the soil above may be dislodged by the force of movement of the ground water escaping into the pit. Miners locally, therefore, may be more exposed to infection at that time. After the flow of inrushing ground water has diminished, the soil above is less likely to be disturbed. At a later period elements of slime in water, oozing from the soil, may settle and grow on the roof to form a nidus for the growth of the leptospira and other organisms. This slime may possibly drop on to the ground, into stagnant pools, and running pit water, from which sources man and rat alike, locally and at a distance, may conceivably become infected. It may be, however, that the pathogenic leptospira escaping from this rich natural organic medium into running water, or water with little organic matter, becomes attenuated after residence in the less favourable environment, as do the human and rat/

rat strains in culture medium. There will be a wider distribution of the organism in infected water than in the slime, therefore dosage may be an important factor in infection. Conditions in the mine which may possibly influence the pathogenicity of the leptospira are darkness, temperature, and depth. The temperature of the slime in the mine was 3°C. higher than that of pumped water in a cement surface pond. No cases of jaundice appeared to be associated with either the pond or an adjacent stream into which the pond water flowed. These waters both showed the presence of certain spirochaetes, but no leptospirae were found in the centrifugalized deposits. Animal experiments with these and other surface waters have so far given negative results.

The opinion stated above, that the absence of favourable and adequate organic matter in the water may be a factor in the attenuation of a pathogenic leptospira, was confirmed by Toyama (1924). In an English version of this author's work, it is recorded that a pathogenic strain actually became attenuated in experimental ponds with no soil, but preserved its virulence for a longer period in ponds with soil. Moreover, it is stated that, "the spirochaete could preserve its poisonous nature two months in an imitation paddy field, which had entirely no self-cleaning function"./

function". The organic properties of the soil and water in which the leptospira may exist as a pathogen would therefore seem to be a factor in the preservation of its virulence. That the presence of the associated organisms in the slime and soil may lower the resistance of man and experimental animals, and so act as aggressins, requires consideration. A French writer, however, inoculated large doses of impure cultures of a water leptospira into guinea pigs with negative results.

Spirochaetes, now considered similar to the genus Leptospira by Noguchi, were observed in water by Wolbach and Binger in 1914. They reported the isolation of a filtrable spirochaete from the shore of a fresh-water pond near Boston. Its pathogenicity for experimental animals was not tested. Several observers since then have recorded the occurrence of the same genus in water. Zuelzer in 1922, discovered in tap water spirochaetes which morphologically resembled those of Weil's disease. Direct inoculation of the naturally infected water into animals, however, did not give rise to any symptoms. One strain of a water leptospira proved pathogenic to guinea pigs after cultivation in serum medium for one and a quarter years - that is, mutation from saprophyte to pathogen had apparently occurred. Stevenson (unpublished observation) found a leptospiral organism in London tap water, a finding that has just been confirmed by Hindle (July 1925)/

1925), but no guinea pig inoculations were carried out. Rats injected with the water gave negative results, (Stevenson). Both fresh and salt water have been found by Noguchi to contain leptospirae indistinguishable from those producing disease in man. The organisms did not prove to be pathogenic to guinea pigs.

Small epidemics of spirochaetal jaundice occurred in the autumn of 1923, in Northern France, one of which was associated with the river Vesle, at Reims. A number of young people who bathed in the river contracted the disease. Etchegoin examined the water and mud of the Vesle, and found a leptospiral organism which he considered had slightly wider elementary spirals than Leptospira icterohaemorrhagiae. The river water and mud, and massive doses of impure cultures of leptospira, were tested by animal inoculation, but did not produce spirochaetal jaundice.

It would thus seem that leptospiral organisms exist as saprophytes in certain water and mud. The saprophytic and pathogenic forms are stated by most observers to be morphologically indistinguishable. Their close relationship in other directions has been demonstrated by Uhlenhuth and Zuelzer. Mention of mutation from saprophyte to pathogen has already been made. Biological and immunological tests carried out by them support the view of the water origin of Weil's disease./

disease. On the other hand, Akira Shiga recently recorded serological differences between certain water strains and Leptospira icterohaemorrhagiae. The latter is stated to thrive in the immune serums of the former, but not in its own immune serum. The two different races of water leptospira tested were likewise only affected by their homologous immune serum, both being uninfluenced by the immune serum of the pathogenic strain. They also proved the greater power of resistance of the water organisms to ultraviolet rays and other influences, but found no great difference in the behaviour of the saprophyte and pathogenic strains towards certain parasiticides.

It has not been possible so far to carry out comparative serological and certain other tests with the slime, rat, and human strains in the present investigation. The most important property of pathogenicity, however, has been proved by animal experiment to be common to the three strains. Natural habitats of the genus have been found in wet soil and in water. It remains to determine if varying natural environments alone can influence mutation from a pathogenic to a saprophytic type and vice versa, or if immutable species exist. The change from virulent to an avirulent organism in altered natural conditions was/

was suspected, and the view held finds confirmation in Toyama's work already quoted. No mention is made of a return of virulence on introducing the water-attenuated leptospira into wet soil again. In the outbreak investigated by the writer it was observed that no cases were associated with the surface pond or stream, both of which received pumped water, presumably containing some leptospirae from the infected pit. The pond was lined with cement, contained little soil, and the temperature was 8°C. - factors possibly favouring attenuation. The stream was said to receive boiling water from mines near its banks. On the other hand, the change from saprophyte to pathogen in natural conditions has not been determined. A water saprophyte, however, was reported by Uhlenhuth and Zuelzer to have acquired pathogenic properties in culture medium.

The localization of the disease has been generally recognized, in spite of the presence of leptospiral organisms in many waters and rats in districts where the disease has not occurred. Epidemics have invariably been associated with wet and badly drained broken ground, as instanced by outbreaks among workers in rice fields and wet mines. During the war epidemics among soldiers were localized more or less to wet,

ill/

ill drained trenches. The disease has also occurred among workers in sewers. In such environments conditions no doubt exist for the development of slime similar to that found in the mine. The slime medium apparently affords an ideal nidus for the growth and aggregation of pathogenic leptospirae. Apart from environment such as favourable wet soil and slime, the organism may become attenuated as mentioned above, which suggests a possible reason for the localization of the disease.

Swimming baths in the poorer quarters of Paris have been held responsible for recent outbreaks, and certain bathing pools in Germany have been incriminated. It may be that leptospirae attenuated by sojourn in water, borne from soil, gain entrance to the ponds along with primitive vegetable elements capable of forming a zoogloea mass as a nidus. In this organic medium exaltation of virulence may possibly occur. One instance has been referred to in which a saprophytic water leptospira became pathogenic.

It is highly probable that rats inhabiting wet mines originally become infected from broken soil. They may acquire infection by feeding on slime in old disused tunnels blocked up with loose stones, as seen in/

in the mine visited. One rat was actually observed to scamper into this ideal hiding place. These old damp, inaccessible branches no doubt afford favourable situations for the collection of much slime, the development of which will probably depend on the nature of the soil above. One means of combating the rat source of infection may perhaps be accomplished by completely walling off such conceivable breeding grounds from the main roadways.

Rats far distant from infected mines in East Lothian were also found to be infected with the leptospira, but no cases of jaundice have been reported in the particular areas. Foulerton, Balfour, and Stevenson recorded the presence of pathogenic leptospiras in rats from different counties in England where the disease was unknown. Coles, at Bournemouth, noted the existence of infected rats there, but pathogenicity tests were not done. These findings suggest that the leptospira is more ubiquitous in soil than at present suspected, and that rats may find a food delicacy in infected slime in soil. It may be also that they can acquire infection from water-attenuated leptospirae which, by passage, become pathogenic to man in certain circumstances.

Reasonable/

Reasonable evidence for the assumption that rats become infected from slime, soil, or water, and so act as secondary disseminators of the disease, was found on examination of their urine. In addition to the presence of leptospirae, similar spirochaetes to those found in the slime and certain waters were found in the urine of many rats by dark-ground illumination.

The work on water leptospirae is still incomplete, but the following tables are presented to convey an idea of the ubiquity of the organism in nature. It was found by the writer in large numbers, particularly in the fungal slime in a number of collieries; and in local reservoir, loch, and sewage water, leptospirae were present but not numerous.

The reaction of the soil and water was ascertained in many instances and it will be noted from the tables that no leptospirae were found in a distinctly acid environment, (p. 119, water samples Nos. 42 and 52a). Naturally acid soil is notably inimical to the existence of the organism, which fact was also recorded by Toyama (1924). An important observation was recorded by Ido, Hoki, Ito and Wani (1917) : they found that the soil and water were of an alkaline reaction in those coal - mines where 80 - 100 cases of the disease occurred anually, and that in mines where few or no cases occurred the soil was acid.

SAMPLES OF WATER FROM BELLYFORD COAL MINE, EAST LOTHIAN.

DATE	No. WATER SAMPLE	SOURCE	REACTION	LEPTO-:SPIRAE PRESENT	RESULTS GUINEA PIG INOCULATION.
1924. Feb. 25th	1	Roof water	pH = 7·6	-	Negative.
"	2	Roof water	pH = 7·6	-	"
"	3	Pool on ground, in pit.	pH = 7·5	-	"
"	4	Surface pond water	pH = 7·5	-	"
"	5	Bellyford Burn	pH = 7·8	-	"
"	6	Roof slime, pit.	pH = 7·4	++	"
"	7	Roof slime		-	"
"	8	Slime from pit props.		-	"
March 14th	9	Surface pond water	pH = 7·5	-	"
"	10	Roof slime	pH = 7·4	+++	2 G.P. inoc$^{d.}$ (15:3:24 with 4 c.c. of slime: both died of typical spirochætal jaundice and numerous lepto-:spirae were found in the tissues.

SAMPLES OF WATER FROM OXENFORD COAL MINE, EAST LOTHIAN.

DATE	No. WATER SAMPLE	SOURCE	REACTION	LEPTO-:SPIRAE PRESENT	RESULTS GUINEA PIG INOCULATION.
1924. Oct. 10th	11	Roof slime.		-	Negative.
"	12	Roof slime, roof bar.		+	"
"	13	Roof slime.	pH = 7·1	++	"
"	14	Roof bar.	pH = 7·1	+	"
"	15	White fungus, wall.		-	No animals inoculated.
"	16	Roof water.		-	
"	17	From Section 1.		-	
"	18	Black slime, Roof bar.		-	
"	19	Roof water		-	
"	20	Roof bar.		-	Negative.
"	21	Stagnant ground water.	pH = 7·5	-	"
"	22	Water and mud, Bellyford Burn.	pH = 7·1	-	"
"	23	Water pumped from pit.		-	"
"	24	Water from "face".		-	"
"	25	Pit water.		-	"
"	26	Burn and pit water mixed.	pH = 7·6	+	"

SAMPLES OF WATER FROM WOOLMET COLLIERY, EAST LOTHIAN.

DATE	No. WATER SAMPLE	SOURCE	REACTION	LEPTO-:SPIRAE PRESENT	RESULTS GUINEA PIG INOCULATION.
1924. Dec. 10th	27	Roof slime	pH = 7·0	+	Negative
"	28	No.90 refuge hole. slime	pH = 7·2	+	No animals inoculated.
"	29	No.92 " " "	pH = 7·5	+	
"	30	No.93 " " "	pH = 7·4	-	
"	31	No.96 " " "	pH = 7·2	++	Negative.
"	32	No.97 " " "	pH = 7·2	++	"

SAMPLES OF WATER FROM PHILPSTOUN SHALE MINE, WEST LOTHIAN.

1924. Nov. 14th	33	Disused section	pH = 7·5	+	Negative.
"	34	Roof water	pH = 7·4	-	No animal inoculated.
"	35	White fungus	pH = 7·5	+	Negative.
"	36	Ground water at dam pipe.	pH = 7·5	+	No animals inoculated.
"	37	Ditch water	pH = 7·4	-	
Nov. 18th	38.	Pond water & "Feed" water		-	
"	39	Slime, return airway.		-	
"	40	Disused pond water		+	
"	41	Stagnant water		+	Negative.

SAMPLES OF WATER FROM DONIBRISTLE COAL MINE, FIFE.

DATE	No. WATER SAMPLE	SOURCE	REACTION	LEPTO-:SPIRAE PRESENT	RESULTS GUINEA PIG INOCULATION.
1924. Nov. 18th	42	Slime from roof timber.	very acid pH = 5·6	-	No animal inoculated.
	SAMPLES OF WATER FROM BALGONIE COLLIERY, FIFE.				
1924. Dec. 23rd	43	From pit	pH = 7·6	+	Negative.
"	44	"	pH = 7·5	-	No animal inoculated.
"	45	"	pH = 7·8	+	Negative.
"	46	" (slime)	pH = 8	++	"
"	47	"	pH = 8	-	No animals inoculated.
"	48	"	pH = 7·8	-	
"	49	"	pH = 7·6	-	
"	50	"	pH = 7·6	-	
"	51		pH = 7·8	-	
	SAMPLES OF WATER FROM KINGSHILL COLLIERY, LANARKSHIRE.				
1925. Jan. 7th	52	Slime from roof	pH = 7·8	+++	Negative.
	SAMPLES OF WATER FROM COAL MINE, ROSLIN.				
1925. Feb. 6th.	52a	Slime from roof	pH = 3·0 very acid	-	No animal inoculated

SAMPLES OF WATER FROM RESERVOIRS, EDINBURGH.

DATE	No. WATER SAMPLE	SOURCE	REACTION	LEPTO-:SPIRAE PRESENT	RESULTS GUINEA PIG INOCULATION.
1925. Jan. 14th	53	Top zoogloeal layer		-	Negative.
"	54	Zoogloeal washings, Settling tanks.		+	"
"	55	Zoogloeal deposit, Settling tank.		+	"
"	56	Do.		+	"
		SAMPLES OF WATER FROM LOCHS, EDINBURGH.			
1925. March 3rd.	57	Duddingston Loch		+	Negative
"	58	Dunsapie Loch		-	No animals inoculated
"	59	St.Margaret's Loch		-	
		SAMPLES OF WATER FROM KIDLAW RESERVOIR, EAST LOTHIAN.			
1924. April 7th	60	Reservoir water		-	No animals inoculated.
"	61	Deposit, Reservoir		-	
"	62	Water trough		?	

Experimental Modes of Infection.

The following experiments were carried out in guinea pigs, to determine possible modes of infection:-

Feeding experiments:

Three animals were fed by means of a pipette with 2 c.c. of heavily infected liver emulsion prepared from a guinea pig.

1.	Fed 8:12:24	Died 4:1:25	No signs of disease: no spirochaetes found.
2.	" 5:1:25	-	Remained alive and well.
3.	" 4:2:25	Died 14:2:25	Suggestion of blood in stomach: no spirochaetes found: considered negative.

Infection by nasal mucosa:

Two animals received a small quantity of infected liver emulsion into nostrils.

1.	8:12:24	Died 10:12:24	Negative.
2.	18:12:24	" 27:12:24	Positive.

Infection by the eye:

In two animals 1 drop of infected liver emulsion was introduced into one eye.

1.	8:12:24	Died 17:12:24	Positive.
2.	17:12:24	" 23:2:25	Positive.

Infection by the unabraded skin:

The abdomen of three animals was shaved and a small quantity of heavily infected liver emulsion was placed on the shaved surface and left there 10-15 minutes.

1/

1.	8:12:24	Died 23:2:25	Negative.
2.	5:1:25	" 19:1:25	Lung haemorrhages only: no spirochaetes found.
3.	4:2:25	" 13:2:25	Positive.

Infection by abraded skin:

Two animals were similarly prepared as above, but in addition, the skin surface was slightly abraded with the point of a scalpel without causing much effusion of blood. A small amount of heavily infected liver emulsion was smeared over the area and left on 5 minutes.

1.	8:12:24	Died 18:12:24	Positive.
2.	4:2:25	" 12:2:25	Positive.

The paw of another animal was scratched to the effusion of blood and a trace of infected liver emulsion applied.

1.	4:2:25	Died 12:2:25	Positive.

One animal received injection of infected liver emulsion per rectum.

1.	4:2:25	Died 12:2:25	Positive.

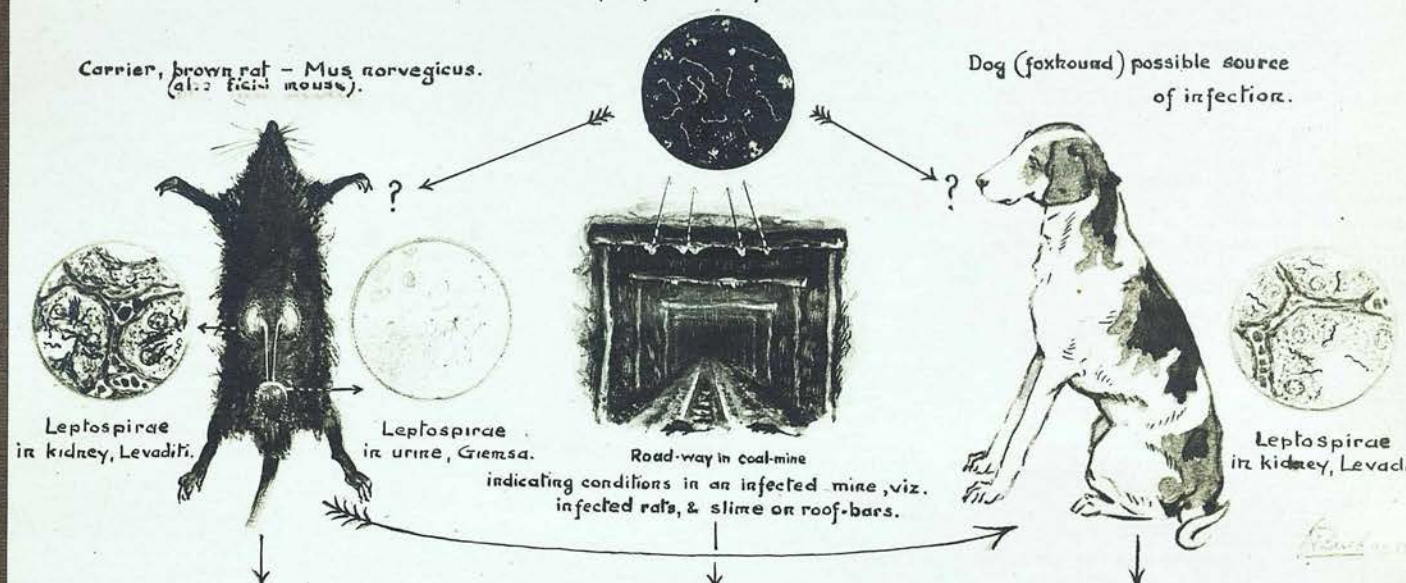
Control animals were injected on each occasion and all died of typical spirochaetosis.

With the exception of the results obtained in the feeding experiments, my experiments compare favourably with those of the Japanese and French writers on this subject. They, however, reported considerable success in infecting animals by causing them/

them to ingest infected liver emulsion. My results in this respect accord with those described by Handel, Ungermann and Jaenisch (1918), who stated that feeding experiments do not succeed as a rule. They also remarked that feeding with infected tissue did not protect against infection by a later injection of "culture".

SPIROCHAETAL JAUNDICE. AETIOLOGY.

(Spirochaetosis Icterohaemorrhagica.)



Several cases in Scotland amongst workers near refuse dumps, in piggeries, and breweries – rats from these sources found infected.

Numerous cases in "wet" coal-mines, Scotland – coal-miners liable to infection from two possible sources – infected rats & slime.

"Yellows" in dogs (foxhound) recently found to be due to leptospiral infection: certain cases in Germany associated with infected dogs.

MODE of INFECTION: infected rat urine → contamination of soil → hands (abrasions) possibly from hands to mucous membranes, mouth, nose, eyes, penetration of spirochaete through intact wet skin of feet possible (miners).

Fig. 45.

V. BACTERIOLOGICAL DIAGNOSIS.

1. Direct examination and animal inoculation of blood.

The direct examination of blood for the spirochaete in human cases of the disease, was found to be of little value as a practical method of diagnosis.

Inada reported their detection in the blood under the dark-ground microscope, in only two out of one hundred cases, during the first few days of illness. Few other records of success in diagnosis either by means of the dark-ground method, or examination of stained films, have been reported. The spirochaetes are stated to be present in the blood, and in very small numbers during the first seven day of the illness. In this investigation the blood of only three patients was directly examined during the first week of illness but with negative results. The disease in the other nineteen patients was not suspected or reported to me until the infective period of the blood had passed; nevertheless direct examination of the blood was done in each case but no spirochaetes were found. Owing to the simplicity, however, of dark-ground examination, the search for the spirochaete in the blood during the early stages of the disease is worthy of trial.

The/

The inoculation of guinea pigs with 4 -5cc. of blood from suspected cases has given satisfactory results in the hands of a number of workers. According to Inada, Ido, Hoki, Kaneko and Ito, and Stokes, Ryle and Tytler, inoculation of guinea pigs with suspected blood, withdrawn on the 4th day of illness, gave 100 per cent positive results. Previous to and after the 4th day of the disease the percentage of positive results was lower. Both the Japanese and British observers quoted, recorded animal infection in a few instances with blood withdrawn on the 7th day of illness, and the former reported one similar success with blood on the 9th day.

The conclusion arrived at by Inada and his coworkers was that animal inoculation with suspected blood must be performed within the first seven days of the illness in order to ensure any degree of success. Intraperitoneal inoculation was recommended, and the animals were stated to have succumbed in 5 - 8 days with typical signs of spirochaetal jaundice.

The writer did not succeed in transmitting the disease to guinea pigs by intraperitoneal inoculation of suspected blood, from one patient on the 5th day, and from another two on the 7th day of illness. These were the only three cases reported to me during the infective/

infective period of the blood; the other nineteen patients were not suspected to be suffering from the disease until after the first week had passed, consequently the opportunity to carry out a favourable blood examination did not present itself.

2. Examination of Urine for the Spirochaete.

It was established by the Japanese observers that the spirochaete usually disappeared from the blood about the 7th day of illness, localised itself in the liver and other tissues, and that from the 9th day up to about the 40th day of the disease the spirochaete could be demonstrated microscopically in the urine.

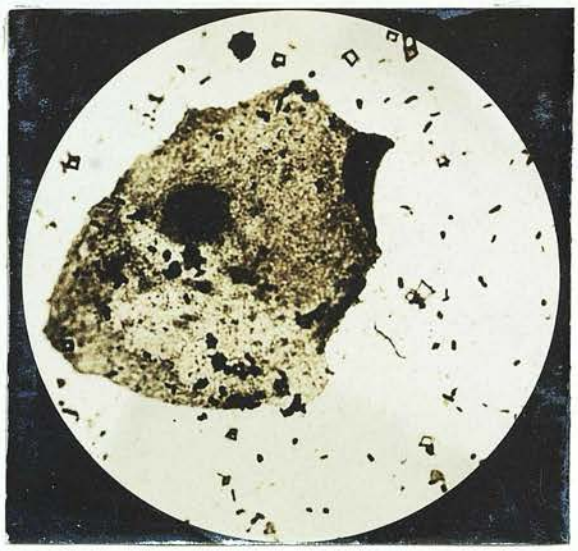
In the present investigation, as the direct examination of blood, and animal inoculation with it proved negative in all cases, microscopic examination of the urine for the organism, and animal experiments with the urine were the further methods employed in attempting to make a bacteriological diagnosis.

Films of the centrifugalised deposit were examined by dark-ground illumination, stained films were prepared, and about 4c.cm. of urine with deposit were inoculated intraperitoneally into guinea pigs and occasionally mice.

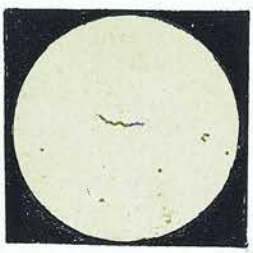
The/

The results of the microscopic examination of the urines after the febrile period, though positive in 17 cases, were not unequivocal. Although spirochaetes were found in the urine of these patients at one time or another they appeared to be devitalised and degenerated. An explanation of this may be found in the acid reaction of the urine and the presence of bile in it, for experiments showed that these properties were not only inimical to the vitality of the leptospira but also destructive to its characteristic morphology. Hence there was difficulty in establishing a diagnosis on microscopic examination of the urine alone. In two of the patients (Cases 10 and 11), seen from the onset of illness, specimens of urine were frequently examined to ascertain the day on which spirochaetes first appeared in the urine. Definite, though few, spirochaetes were first found on the fourteenth day in Case 10, and on the twelth day in Case 11. In the other cases circumstances only permitted of obtaining the first specimen of urine at varying dates after the acute stage. Spirochaetes were found in the urine of the 17 patients, between the eighteenth and twenty-third day of illness. With the exception of Case 11 spirochaetes were not observed in the urine after the thirty-seventh day. In each instance spirochaetes were detected as a rule with difficulty; exceptionally three or four might be encountered/

Case 19. M.Mc. Fontana's stain



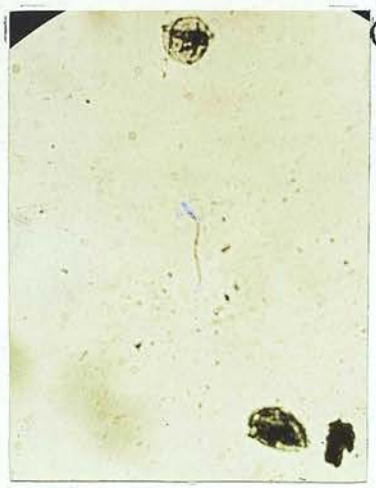
Case 11. M.B.. Giemsa's stain.
(Guinea pig inoculation +)



Case 20. A.McD.. Fontana.

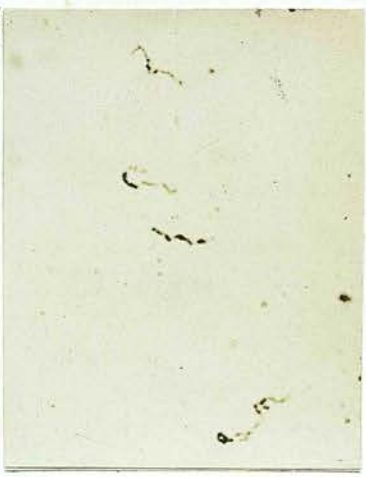
Case 1. T.W.. Fontana.
(Guinea pig inoculation +)

Case 12. J.K.. Fontana's stain.



Case 6. B.W.. Giemsa's stain.

Case 9. N.B.
Fontana's stain.



Case 7. R.E.. Fontana's stain.

3. Micro-photographs of spirochaetes in stained films of urine from eight cases of spirochaetal jaundice (vide table p.16). The organisms were not recognisable with certainty from saprophytic spirochaetes, except that in some instances the granular appearance suggested the fine spiral structure of altered leptospirae. Inoculation of two of the urines (Cases 1&11) reproduced spirochaetal jaundice in the guinea pig.

encountered in one microscopic field and none on further examination of the slide. By dark-ground illumination the fine spirals were not recognised, but they seemed to be indicated by a slight granular appearance, which was more pronounced in the stained films. The forms observed could only be regarded with suspicion as of the leptospiral type, so that it remained to rely on the results of animal inoculation for confirmation. Repeated failure to produce by this means any signs or symptoms of the disease in animals prevailed over a long period and necessitated the use of a large number of animals. The devitalising effect on the leptospira of the acid and bile in the urine was held partly responsible for the failure to infect. Although bile ceased to be excreted in the urine after a time the reaction remained strongly acid. The difficulty of confirming the urinary observations by animal inoculation will be more readily appreciated by reference to experiments associated with a local out-break of the disease. Forty-four animals, including four mice, were employed before absolute confirmation of the clinical diagnosis was obtained. Thirty-one of these animals remained alive and well and 13 died; the latter number comprised 11 guinea pigs and 2 mice. The results of the animal inoculations with the urines obtained at different periods during the course of illness/

illness may be tabulated as follows:-

Table Showing Results of Animal Inoculations, with Urine of Patients.

Case	Animals Inoculated.	Survived.	Died.	Result. Post-mortem findings. Jaundice.	Lung Haemorrhages.	Leptospirae.
12	2	2	0			
8	5	5	0			
9	6	5	1	—	+	—
10	11	9	2	—	—	—
11	11	6	5	(1 + (2 — (3 — (4 — (5 —	+ + + — —	+ — — — —
13	2	1	1	—	+	—
14	4	1	3	(1 — (2 — (3 —	+ + —	— — —
15	3	2	1	—	+	—

The outstanding feature in the table is the record of only one definitely positive result out of 44 animal experiments. Seven of the other animals showed "spotted" lung haemorrhages, which lesion is considered a most important diagnostic sign of the disease in experimental animals (Inada).

Noteworthy observations are associated with the urine/

urine obtained from Case 11, and with the infected animals. Spirochaetes were found in the urine of this patient on six different occasions and up to the sixty-sixth day from the onset of illness. Examination of the urine on the sixty-sixth day revealed the presence of only a few spirochaetes, and the animal inoculated with this specimen succumbed in 30 days with signs of typical spirochaetosis. The fatal issue was singularly postponed, but the paucity of the spirochaetes in the urine, amongst other factors, may be a cause of this; inoculation of infinitesmal doses of culture may not prove fatal until about the twenty-ninth day. Subinoculations into other guinea pigs from the originally infected animal have given very irregular results, pointing either to variability in the selective action of this strain of leptospira, or to individual idiosyncrasies in the animals inoculated. In the third animal passage both jaundice and skin haemorrhages were absent, but the lungs were typically mottled; spirochaetes were found with difficulty in the liver. In the fourth animal passage the only macroscopic lesion was confined to the uterus, the whole of which presented the appearance of a bicornuate blood-clot. But for this lesion the animal showed no naked-eye evidence of infection. Here again spirochaetes were extremely few/

few in the liver or other organs, and none were found in the uterine blood. These facts are mentioned merely to indicate the peculiar behaviour in animals of this human strain. So far the passage of local rat strains through guinea pigs has given, as a rule, more uniform results.

Similar difficulties have been encountered by other workers, (Inada etc., Garnier and Reilly, Cappellani and Fragoni, and Martin and Pettit), both regarding the recognition of the spirochaete when in the urine, and the failure of such urine to infect guinea pigs. Several of these writers reported that two thirds of the positive urines, some even containing numerous spirochaetes, failed to produce infection in animals.

Noguchi considered that an explanation may be found in the possible attenuation in virulence of the organism during the course of infection, and also noted that strongly acid urine, and the presence of bile in it were inimical at the end of 24 hours. My own experiments with urines strongly acid and containing bile, demonstrated the rapidly distructive action of these properties on the living leptospira under the microscope, in a very short time.

The conclusion arrived at from the practical point of view, is, that in a case of jaundice the clinical/

clinical features of which suggest the spirochaetal type, and in the urine of which granular forms of spirochaetes are found, the chain of evidence is strong enough in my opinion to justify the diagnosis of spirochaetal jaundice.

4. Immunity.

It was reported by the Japanese observers (1916), that the blood serum of patients convalescent after the disease possessed properties inimical to the spirochaete, and capable of protecting guinea pigs from infection. They noted that the immune substance was demonstrable after the 10th day, but as a rule was not found before about the 15th day, at which time spirochaetes usually became numerous in the urine. It was stated that this substance was specific and not present in the serum of healthy persons, or in cases of jaundice of other origin. By the application of Pfeiffer's test, performed by intraperitoneal inoculation of the guinea pig with liver emulsion rich in spirochaetes, and with patient's serum, they found that the peritoneal fluid was free from spirochaetes after ½ - 2 hours, whereas in the control animals they were present. Curative properties were also demonstrated by the inoculation of serum from a convalescent subject into an infected guinea pig. The spirochaetes disappeared from the blood after half an hour; on sacrificing the animal after 3½ hours, only degenerated forms were found in the liver, and after 8 hours spirochaetes were/

were observed only in the kidney and suprarenals.

These observations have been confirmed by Martin and Pettit, Stokes and others.

The French writers, Costa and Troisier, demonstrated the presence of complement-deviating property, "sensibilisatrice", which, however, was not specific. They stated that a positive reaction was obtained with a syphilitic antigen and the serum from a case of spirochaetal jaundice, and that with an antigen prepared from the liver of a guinea pig with typical spirochaetosis, a positive result was obtained with sera, both from cases of syphilis and spirochaetal jaundice.

Martin and Pettit described the presence of agglutinins, usually demonstrable about the 10th day, which permitted the application of the Widal test. This in my opinion is not a reliable aid in diagnosis, as I was unable to demonstrate a definite reaction with the sera from several moderately severe cases of the disease. According to Garnier and Reilly the patient's serum does not agglutinate the spirochaete. A Japanese observer, Oba, working with rabbits stated that agglutinins were clearly demonstrable in the animals blood for over four months.

Lytic properties of the serum in some of the writer's cases were distinctly demonstrated under the/

the dark-ground microscope, and proved more reliable as an aid to diagnosis than the agglutination reaction. This view was also expressed by another writer who remarked that spontaneous agglutination of the spirochaete was liable to be misinterpreted.

In view of the facts ascertained in 1916 by the Japanese workers they prepared an anti-serum by immunizing a horse, and reported favourably on the use of this serum therapeutically. Martin and Pettit at a later date also reported satisfactory results with anti-serum prepared by immunizing both the rabbit and the horse.

The writer was instrumental in getting the first anti-spirochaetal serum prepared (1924) from the Scotch strain of spirochaete. This was effected by providing a primary culture from an infected local rat; the horse was immunized and a fairly potent anti-serum was obtained. So far it has only been possible to test its efficacy in four human cases; in two the temperature dropped on the 5th day from 102°F. to normal after the intravenous administration of 20c.c. of anti-serum on the 4th day of illness. In one of these cases (No.10), however, severe haemorrhages occurred at a later date but the patient recovered.

The few cases treated by anti-serum administration/

administration within the first seven days of illness, do not permit of forming a definite opinion regarding the efficacy of this particular anti-spirochaetal serum.

VI. Prophylaxis.

Active immunization by injection of a vaccine of the spirochaete was experimentally proved by Ido, Hoki, Ito and Wani. Ito and Matsuzaki at a later date actively immunized the human subject by subcutaneous inoculation of 2.5cc. of a vaccine prepared from cultures of the organism. In a report by Wani (1919) better results were obtained by administering two doses of vaccine, first 2cc., then 3cc. after a period of a week. Three weeks later the serum of the eight subjects treated was examined for Pfeiffer's reaction which was stated to be positive in all the eight cases. Prophylactic injections of vaccine were employed on a large scale in Japan with successful results.

Toyama (1924) advocated the use of calcium cyanamide (18.78 kilograms per 991.73 square metres) in/

in agricultural regions where the disease was endemic.

This substance did not interfere with the growth of the rice plant and in the endemic regions where it was applied to the soil no further cases of the disease were reported. Lime nitrogen was also used (41 lbs to ¼ acre), at an earlier date (1920) with equally striking results.

The extermination of rats, as far as possible, both in coal mines and generally, constitutes an important preventitive measure.

Inada stated that by improvement of the drainage in wet mines the number of cases amongst coal-miners in Japan decreased.

Although no cases of contact infection amongst guinea pigs have been recorded, which fact has been confirmed by the writer, nevertheless human outbreaks have occurred suggestive of this mode of infection, therefore the usual precautions with urine, stools and other excreta should be put into force.

Insects have not been incriminated as carriers of the virus.

SUMMARY.

The jaundice, which occurred among coal-miners working in certain collieries in East Lothian, was diagnosed clinically by Professor G. Lovell Gulland, and proved bacteriologically by the writer, to be of spirochaetal origin (January 1924).

Wild rats and field mice from the infected mining area were incriminated as carriers of the causal organism in Scotland for the first time (January 1924).

The morphology of the organism was found to be distinctive, and different from ordinary types of spirochaetes described. It agreed in structure with the organism first discovered by Inada and his co-workers (1914), who named it "spirochaeta ictero-haemorrhagiae", and thereafter, they introduced the title of "spirochaetosis icterohæmorrhagica" for the disease. The features of the organism consist in the presence of fine spirals throughout its length, and incurved ends. Noguchi (1917), on account of its unique morphology, suggested a new generic name for it, viz. - Leptospira, and the organism is referred to by various writers as spirochaeta or leptospira icterohaemorrhagiae. The distinctive structure was more readily and easily demonstrated/

demonstrated by means of the dark ground microscope.

The existence of the disease among members of the community in Scotland, other than coal-miners, particularly in those working in rat-infested areas, e.g., near refuse dumps, piggeries and in breweries, was proved at a later date.

The diagnosis in 22 cases of the disease was based on the combined clinical, laboratory and experimental observations described.
In 22, jaundice was a feature.
In 17, haemorrhage, mainly epistaxis, occurred.
In 17, spirochaetes were found in the urine.
Spirochaetal jaundice was produced in two guinea-pigs inoculated with urine from two of the patients, and in eight "spotted" lung haemorrhages were the only signs of infection.

The death rate among those who suffered from the disease was found to be as follows :-

Coal-miners	17	5 died	mortality = 29%
Other occupations	14	3 died	mortality = 21%
Total, all occupations	31	8 deaths	Mortality for Scotland = 25%

The figure for the death rate among coal-miners approximates very closely that reported by Inada in Japan, viz. - 30·6 per cent.

The/

The notable findings at autopsy consisted of generalised intense yellow colour of the integuments, skin petechiae, and haemorrhages, which were observed, at one time or another, widely distributed throughout the body and particularly evident in the stomach.

The microscopic lesions of note confined themselves chiefly to the kidney and liver. The kidney invariably showed marked degenerative changes and areas of necrosis were present in some instances. The liver was not so profoundly changed as the kidney but areas of cell necrosis and focal accumulations of cells were observed. Chauffard applied the name of "hepato-nephritis" in cases where equally pronounced degenerative changes occurred in both organs. Otherwise, capillary haemorrhages were a feature in most of the tissues, and phagocytosis of red cells occurred, particularly in the lymph glands and spleen. Spirochaetes were difficult to find in the tissues.

The disease in experimental animals invariably assumed a rapidly fatal course, with death in 5-12 days after inoculation. The initial signs of the disease in infected animals find a parallel in those signs associated with human infection as a rule, but blood infection is more readily demonstrated in animals than in man.

Blood examination of infected guinea pigs revealed/

revealed a new observation, viz. - the presence of a relative small lymphocytosis within the first few days after infection. The presence or absence of this in suspected human cases during the initial stage of the disease might furnish an additional aid to diagnosis. A pronounced terminal anaemia was also noted in experimentally infected animals.

The pathological features in the guinea pig, although in the main comparable to those observed in fatal human cases, were much more pronounced and extensive. Contrary to human infection, spirochaetes were found with ease in the blood and in practically all the organs at death. The lung lesions in the guinea pig were characteristic of spirochaetal infection, and presented a "spotted" or "butterfly-wing" appearance. This picture was not appreciated in the human lungs from any of the fatal cases. The kidney and liver were the seat of marked degenerative changes, just as may occur in severe and fatal infection in man.

Both atypical signs of infection during life, and pathological changes at death, were noted in several infected animals.

Certain writers suggested that the existence of a peri-cholangitis in both human and animal forms of the disease might account for the jaundice; others incline to the view that it is of haematogenous origin. The writer/

writer has stated reasons, based on experimental findings, for concluding that the occurrence of jaundice within the first few days of illness is not of haematogenous origin.

The morphological features of the organism both in the living form and in tissues, and the cultural conditions of growth, have been studied and described. The virulent organism was found to survive, when inoculated into certain pit waters and soil, for 95 days. It proved non-pathogenic when inoculated into a guinea pig at that time.

The investigation into the question of carrier hosts of the organism disclosed the following figures relative to the percentage of wild rats infected with the virulent leptospira :-

Total number of rats examined.	Positive	Per cent infected.
166	61	36·7

Among 102 rats from the Edinburgh area, 30 were positive, i.e., 29·4%. One field-mouse also showed the leptospira in the kidney. The organism in these two animal hosts was found only in the kidney or urine.

Experimental modes of infection were investigated in the guinea pig. From the results obtained experimentally, and from the circumstances of work and the condition of the workers in certain coal mines, the writer is of the opinion that human infection is more likely/

likely to occur as a result of the organism gaining entrance to the body through skin abrasions, or by way of the eye and the nasal mucosa from contaminated hands. Infection by ingestion would appear to be a less likely mode of infection in my opinion.

An enquiry into the question of other possible sources of infection in certain coal-mines where the disease occurred, revealed the following original observations :- Leptospiral organisms were dis-:covered in fungal slime hanging from the roof, and in pit and surface waters. Inoculation of a certain specimen of the slime into two guinea pigs produced typical spirochaetal jaundice. Similar organisms of leptospiral type have been discovered in local reservoir, loch, and sewage water and in several other coal and shale mines.

The question of two distinct species of the leptospira have been discussed, viz. - a water or saprophytic type and a pathogenic form. The writer's work regarding a conceivable mutation from saprophyte to pathogen is incomplete, but he is of the opinion that this may possibly occur in certain natural environments.

The establishment of a diagnosis by means of bacteriological procedure was attempted. Blood examination for the spirochaete proved negative in all cases/

cases, as did guinea pig inoculations with suspected blood. The urinary examination for the presence of the spirochaete proved more helpful, but the organism was never observed under the dark-ground microscope or in stained films in typical form. The results of guinea pig inoculation with suspected urine containing spirochaetes were very uncertain. The writer is of the opinion, however, that in cases of jaundice in which the clinical signs point to the spirochaetal form, and in the urine of which granular spirochaetes are found, the chain of evidence is strong enough to justify the diagnosis of spirochaetal jaundice.

As an aid in diagnosis, serological methods were applied in several cases but without very definite results. No agglutination of the spirochaete was observed when tested with the blood serum of four patients during convalescence, but a lytic action of the serum on the spirochaete was demonstrated in a few instances. The serological aspect of human infection was not studied to any extent owing to the difficulty of obtaining material for this purpose. The writer was instrumental in effecting the prepar-:ation of a specific anti-serum from the local strain of the organism. The number of human cases in which it has been used, is too few, however, to state an opinion regarding its efficacy.

The/

The chief prophylactic measure is agreed to be rat extermination as far as possible, and moreover in infected coal-mines, improved drainage, as Inada found that this measure was responsible for a decrease in the incidence of the disease in certain wet mines in Japan. The usual precautions applied to other infectious diseases should also be put into force. Active immunization was established by the Japanese who administered spirochaetal vaccines with very satisfactory results.

The work has been carried out at the Royal College of Physicians Laboratory and at the Bacteriology Department, University of Edinburgh.

I desire to express my thanks to the Laboratory Committee of the College and to Professor T. J. Mackie for facilities afforded.

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